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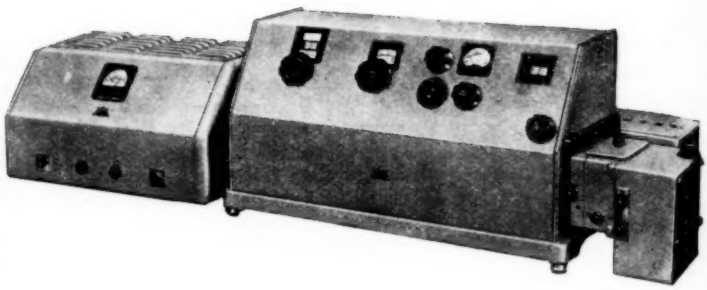

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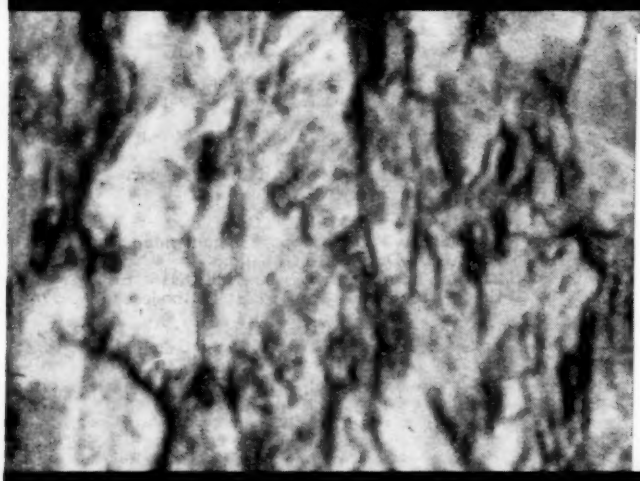

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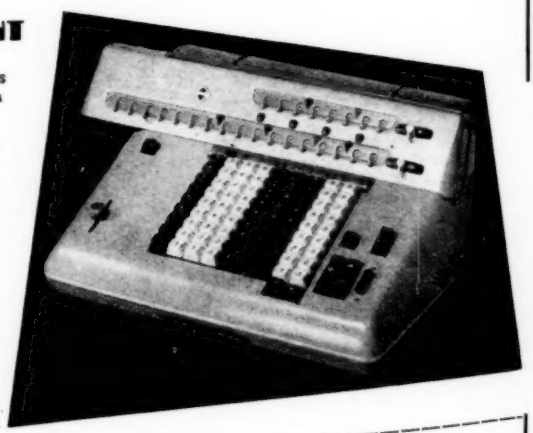

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THE SCIENTIST AND THE COMMUNITY

THE address, "Power and Responsibility in Science", which Prof. M. Swann broadcast in the Third Programme on the eve of the British Association for the Advancement of Science meeting at Norwich (August 29) in no way anticipated Sir Wilfrid Le Gros Clark's presidential address; though in his concluding passages Sir Wilfrid summoned scientists to play a special part in promoting opportunities for free intercourse and the free interchange of ideas among the peoples of the world. Prof. Swann is no less convinced that scientists have something to offer the world besides their discoveries, but he did not touch on this particular question of co-operation, except to note that, in simple terms of its influence on mankind, H. B. du Pont, when addressing the Franklin Institute, once described the development of the modern industrial corporation as the greatest invention of all, exceeding even the influence of the steel plough, steam locomotive, the telegraph and telephone, and the flour mill of Oliver Evans, which was the precursor of automatic control.

Prof. Swann began indeed by referring to certain discoveries of science which had reduced drudgery, transformed communication and transport and enhanced or improved the standard of living, or relieved pain and suffering, doubled our life-span and vastly improved food production. These discoveries, apart from the impact of science on ways of thought, could undoubtedly be placed on the credit side even if there were other things such as the atom bomb or the misuse of scientific ideas to bolster up racial, economic or political theories to be placed on the other side.

Prof. Swann, however, was not attempting to strike a balance but to indicate the vast power which science has come to wield over the human race. This power, he argued, is exercised without responsibility and this is one reason for the anti-scientific feelings sometimes encountered. Moreover, because every scientific discovery depends on what has gone before, such power as science exercises is not applied by individuals but collectively by scientists as a whole and all must in some sense accept responsibility. While at the individual level the concept that power and responsibility must be linked has long been established, its acceptance at the collective level of government and society is a much more recent achievement, and Prof. Swann taxes science with having evaded this issue of the responsible exercise of power which is at the heart of modern democracy.

It was not suggested by Prof. Swann that this evasion is deliberate. It arises out of the very nature of science as, first and foremost, a search for knowledge, and it was much later—Prof. Swann puts it within the past century—that knowledge came to mean power, and still more recently that the power and the attendant responsibility of science became glaringly obvious. To-day, he remarks, even a research worker in the most esoteric corner of science can no longer feel sure that he is not laying foundations for some new advance with all sorts of practical implications. The scientist who fails to develop a social conscience can no longer be excused.

Prof. Swann does not, however, analyse this question of responsibility at all deeply or even the position of the scientist in the community in which he works. He considers the question whether the scientist has anything beside his discoveries to offer the world in which practical decisions have to be made and discoveries translated into action, for good or for ill. He recognizes, of course, that under present conditions society contrives fairly effectively to prevent scientists as such from doing much to improve the situation. Prof. Swann makes the point that there is just as much need, and possibly more immediate prospect of success, for scientists to enter public life vigorously at lower levels than those of the higher reaches of government—in the councils, committees, boards of governors, through which the life of the local community is ordered. Moreover, he suggests that foresight is only a part of the scientist's whole attitude to tradition and progress and that here the scientist has something further to offer to the national way of thought.

Considering the way change occurs in public life, Prof. Swann points out that to the scientist the past is worthy of respect and represents the best that able minds could achieve. The scientist, above all men, depends on the work of those who have gone before him, and because we have the advantage of other men's labours we should be able to go further and do better. Prof. Swann looks on the scientist as a conservative and evolutionary influence in public life rather than a revolutionary influence, seeking to add to the structure, remodel it here and there, to change but not to destroy.

Closely linked with this is the stress Swann lays on the scientist's insistence on the experimental approach. This is built so firmly into science that the scientist scarcely realizes how much less instinctive it is in the outside world, and Prof. Swann frankly advocates an extension of the experimental approach, for example, in such matters as nationalization. Here, of course, he enters on the wide issues of influence, as opposed to power, and of the place of the scientific outlook and scientific method in public affairs, and these are vital aspects of the problem of the right relation of knowledge and power. Moreover, it is necessary to distinguish between the scientist as a scientist and the scientist as a citizen, for the responsibilities are not identical, and failure to

distinguish between them has led to mistaken action as well as to the opposition to science which Prof. Swann notes.

The scientist as such has influence rather than power. It is his responsibility to exercise his foresight so far as in him lies, and to point out the consequences that must follow the adoption of particular scientific or technical measures. Only to that extent does he bear political responsibility as a scientist. Whether, however, he is able to exercise that advisory function at a high enough level or sufficiently effectively or early is a matter for society as a whole, and it is here, as citizen, that the scientist must recognize a responsibility to work to ensure that the structure of government allows scientific and technical advice to be tendered appropriately and effectively. This civic responsibility requires also that he should seek to ensure that educational policy is such that it provides for the task of administration those competent to assess scientific and technical advice and give it due weight along with other factors in a situation.

It is only when scientists as citizens prove effective in the discharge of such responsibilities that one can speak of the scientists as exercising power. Even if, and when, a scientist is appointed to a position of high responsibility in the executive government, it is not as a scientist but as a citizen that he holds and exercises power. It is important that this should be clearly understood, for confusion over the relations between the layman and the scientist to-day is responsible for much of the opposition to the wider use of the scientific method and to the establishment of conditions in which scientific work is most effectively done. It is scarcely conceivable that there will be any reversal, either in the advanced or the backward races, at the national or at the international level, of the process of applying the fruits of scientific knowledge. But the real reason why this confusion over the relations between the layman and the scientist and their respective responsibilities for the use or misuse of science is dangerous was brought out rather better by Sir Solly Zuckerman in the Stephen Paget Memorial Lecture in November 1959, and in his address at the California Institute of Technology in June of that year (see *Nature*, **184**, 135; 1959).

Sir Solly pointed out that there is a sense in which science is the handmaiden of the economic or social institutions in which it functions—a framework which is indeed to some extent continually being transformed by new scientific ideas and the application or practical development of those ideas, but which nevertheless to some extent determines what ideas shall be developed. A material civilization of motor-cars, or jet planes, of radio and television, of man-made fibres and nuclear power is not necessarily the only form that civilization might have taken. Now that it has taken that shape, Sir Solly remarked, it helps to define for us the content and the boundaries of the area within which we exercise our freedom. Pursuit of some major development, for example, the development of the steam engine, means that some other path that potentially might have been followed is not followed.

This is as yet only imperfectly grasped in Britain to-day, though it is being strongly emphasized by such topics as space research. There are, moreover, two further points to be made in this context. First, this choice of development is not one that is made by the scientist as such. It is a political choice, only to a limited extent made on scientific or technical advice, and Sir Solly Zuckerman reminded us that it may not even be conscious, and that only partly because of the unpredictability of any new major scientific advance or the increasing complexity of scientific knowledge in the applied field. It is also partly because in the application of scientific knowledge means tend to become ends, the discovery of new ways of doing things transforming the things done and so their purpose.

Here Sir Solly's vision is clear, but its implications have not as yet been widely discussed or the requisite steps taken to provide some means of guarding against the dangers to which he directed attention. Rejecting as too simple the idea that to-day democracy has abrogated its rights to a bureaucracy which is determining the direction we are taking, Sir Solly maintained that, since the element of the unknown in government increases with every step we are now taking to apply the fruits of science, if sovereign power is being abrogated it is less to a governing body, however formed, and more and more to a process of applying scientific knowledge without any real possibility of determining its final consequences. Neither the voice of the majority nor those through whom it is expressed can proclaim the precise lines of the future.

This is a vital point which Prof. Swann overlooked; it is dangerous to speak of the power and responsibility of the scientist if it is not recognized that while the scientist may be the better able to appreciate the scientific aspects of a situation, he is not responsible as such for the application of the new knowledge and cannot be held responsible for predicting some vast social transformation which might result from a seemingly trivial observation. Nor is it sufficient to urge the need to regard education in science as constituting an essential element in a liberal education. We need above all to appreciate, as Sir Solly put it, that in the major scientific matters which now affect human destiny, we cannot safely take decisions for to-day unless we realize that those same decisions determine the future. It is at this point that the scientist's foresight ought to be exerting its decisive influence, and the survival of mankind may well turn on the urgency and vision and determination which are brought to bear on the creation of the institutions which will enable the scientist to make that contribution effectively. Nor is that solely a challenge to creative thinking or inventiveness. It is equally a challenge to the scientist and technologist to respond to Prof. Swann's appeal and use to the full, imaginatively and constructively, such opportunities of public service that may already confront them as citizens. Even when new opportunities are provided, scientists and technologists will only be able to use them adequately as they gain

experience of public work. Perhaps one of the most constructive steps that our major scientific professional institutions could now be taking would be to stimulate the interest of their younger members in public service and to assist them to gain the experience and the outlook which would enable them to make their particular and unique contribution most effectively.

HUMAN PARASITES

Animal Parasites in Man

By Dr. N. H. Swellengrebel and M. M. Sterman. Pp. x+652. (Princeton, N.J. : D. Van Nostrand Company, Inc. ; London : D. Van Nostrand Company, Ltd., 1961.) 67*s.* 6*d.*

IT is difficult, as one reads through this book, to decide what type of reader its authors had in mind. First issued sixty years ago, it has now been re-written and enlarged, and its authors hope it will be useful to the physician and the biologist. For the physician seeking an introduction to this subject and information about the epidemiology and treatment of human parasitic infections it may well seem adequate; but the biologist or medical man who requires guidance and information on difficult points of detail or procedure may prefer one of the several text-books of medical parasitology which are available at not much greater cost. The biologist, moreover, may wish for more systematics than this book attempts to give and more on the features of the main groups of parasites. He may wonder, too, at some statements, such as the suggestion that all trematodes are flat "like a flatfish or the leaf of a tree"; and why the authors have introduced into an otherwise excellent introduction such terms as "proliferous" and "non-proliferous" parasites, "initial aggressors" and the like ; and at the division of the common parasites of man into the Protozoa, Metazoa and the "Scolecida, or lower worms"; one wonders, too, whether Sabin would nowadays agree with the statement attributed to him in 1953 that toxoplasmosis is an "almost perfect host–parasite relationship in which the parasite only rarely causes serious damage to its hosts, and the hosts are not too intent on destroying the parasite".

Apart, however, from points like these, the text is, as one would expect from a book bearing the highly respected name of Prof. N. H. Swellengrebel, accurate and as up to date as any book can, in these days of very rapid advances in knowledge, hope to be. Among the best sections are the new chapters on methods of examination of the excreta and blood for eggs and larvæ of parasites and on the methods for the enumeration of the eggs of parasites and on serological methods of diagnosis. The bibliographies at the end of each chapter will also be useful to readers who wish to extend their knowledge. The book is written in an easy style and shows the host and parasite in conflict with each other, a point of view which leads the authors to use such phrases as "the host's defense mechanism was kept constantly on the alert" (p. 127), or the host applies "topical" or "numerical" restraint on the parasite, phrases which are useful only if great care is taken not to mislead the reader. An odd feature is the disproportionate space given to the rare occurrence of *Babesia bovis*, a parasite of cattle, in splenectomized human subjects. It is true that babesiasis may be a risk to which man, and especially splenectomized man, may rarely be exposed, and the space given to this disease would be justified in a large and comprehensive text-book ; but it should be accompanied by a fuller consideration of the Babesiidae than is given here. It is difficult, too, to understand the statement (p. 127) that "calves are the only hosts to suffer" from *Babesia bigemina*, because they suffer less severely than adult cattle and it is explained on the same page that they acquire pre-immunity. It is implied, also, that *Boophilus annulatus* is the only invertebrate host of *Babesia bigemina*, whereas at least 10 ticks belonging to 3 genera have been recorded as its invertebrate hosts. The tick, moreover, is here described as the intermediate host, whereas it harbours the sexual phases of the *Babesia* and is therefore the definitive host.

Most of the numerous illustrations are taken from other text-books, and it is unfortunate that many have suffered much in the process of reproduction. Outstanding examples are the figures of *Echinococcus granulosus* (p. 267), and the black-and-white figures of the exo-erythrocytic stages of *Plasmodium inui* and *P. falciparum*, which give no idea of the clarity and draughtsmanship of the originals. These examples are the more regrettable because other figures are well reproduced.

The format of the book and its general make-up and print are attractive, but its price, compared with that of the bigger and more detailed text-books available, is high.

G. LAPAGE

CANCER OF THE RECTUM

Cancer of the Rectum

Edited by Cuthbert E. Dukes. (Neoplastic Disease at Various Sites, Vol. 3.) Pp. xiii+304. (Edinburgh and London: F. and S. Livingstone, Ltd., 1960.) 50*s.* net.

THIS is the third volume of the series *Neoplastic Disease at Various Sites* under the general editorship of Prof. D. W. Smithers. In one way, at least, the volume is unique: eleven of the eighteen contributors are connected with St. Mark's Hospital, London—seven as consultant surgeons, two as consultant pathologists, and two as research assistants. Not only are these contributors familiar with each others' views and methods, but also for many years the work of the surgeons has been co-ordinated through a single pathology department and follow-up system. In this connexion the immense and universally acknowledged contribution of Dr. Dukes himself is further made evident by the fact that his accurate observations, his plan for the examination of operation specimens and dissection of lymph nodes and blood vessels, his pathological classification, and his histological grading of rectal cancer, form the basis on which the contributing surgeons analyse their operative results, and are unquestionably the framework on which many of the recent advances in surgery of rectal cancer have been made.

Theoretically, at least, the fact that so many of the contributors have been working in close association with each other, carries with it the danger that the views expressed here are narrowed through inbreeding. Only in Chapter 13, written by Prof. J. C. Goligher of Leeds, is mention made of methods practised in other countries. Some may be surprised to read that

combined abdomino-perineal excision by two surgeons operating simultaneously is little practised in America, or in Germany and other Continental countries. The views of an American or Continental surgeon, particularly with regard to the choice of operation, might well have made an interesting additional chapter. As it is, the reader is left with the knowledge that the abdomino-perineal excision is the operation most commonly practised in Britain, but with the feeling that anterior resection with sphincter preservation might be the better operation for at least a proportion of cases. On the other hand, one hastens to say that the descriptions of the different surgical techniques are both remarkably clear and admirably brief. Each is written by a surgeon who has immense experience of the operation in question.

Some of the tremendous advances in surgical technique, especially in the resectability-rate, during the past thirty years, are attributable to improved anæsthesia, antibiotics, anticoagulants, a better understanding of surgical shock and its treatment, and to the consequent possibility of undertaking more radical operations. Some have arisen as the direct result of pathological observations, for example, the observation that cancer of the rectum rarely spreads downwards and therefore that sphincter preservation is feasible. Finally, some are undoubtedly the direct result of the concentration of surgical and nursing experience at St. Mark's Hospital itself. In this latter connexion this book is a poignant answer to those who still oppose the establishment of ultra-specializing treatment centres in Britain.

The section (pp. 36–39) by Dr. Dukes on the control of rectal cancer in polyposis families provides an unparalleled example of cancer control. By keeping detailed records of polyposis families and by going to great lengths to keep in close touch with them, it has in many cases been possible to undertake colectomy before metastasizing cancer has developed. At the same time an understanding of the inheritance of the disease has enabled the surgeon to relieve the minds of unaffected siblings of the fear that they too might eventually develop the disease.

Surprisingly, no special space is set aside for consideration of the pre-operative general examination or preparation of patients. On p. 251, in the chapter on operative complications (Chapter 22), Mr. Gordon Ungley writes: " . . . a pre-operative intravenous pyelogram is of immense advantage, should serious urinary complications arise". The desirability of conducting this and other investigations pre-operatively in certain patients should surely have been discussed somewhere earlier in the volume.

As regards alternatives to surgery, cancer chemotherapy is not mentioned, but radiotherapy is well dealt with by Dr. Williams in Chapter 19. Here no strong claim is made for the value of radiotherapy other than as a palliative measure. On the other hand, it is pointed out that only recently have X-ray voltages in excess of 1 MeV. been available; these give less severe skin and mucosal reactions than lower voltage X-rays, and better results may be expected from radiotherapy in the future.

The authors of the final section entitled "Possibilities for Future" (p. 277) conclude that further improvement in prognosis must depend mainly on earlier diagnosis and earlier surgical treatment, but they do not suggest how these may be achieved. It seems unlikely that exfoliative cytology, which is mentioned briefly on p. 132, will be found to have much value as a quick screening test.

One problem which urgently needs an answer is "to what extent, if any, does surgical biopsy, and the pre-operative or operative manipulation of tumours increase the risk of distant metastasis via the blood stream?" Now that appropriate and reliable methods for the detection of circulating cancer cells are becoming available, it is to be hoped that this question will soon be answered. The technique called 'Chemosurgery' developed by Mohs[1] is being increasingly practised in the United States and some European countries. It is likely that it would prove of value in the diagnosis and treatment of possibly malignant polyps and of lesions of the lower rectum near the anal margin.

At the end of an excellent review chapter (Chapter 5) on "Tumours of the large intestine of mammals other than man", Dr. Roberts discusses the experimental induction of tumours of the large gut by chemical substances. The possibility that constituents of the diet, or that anal ointments or suppositories, play an ætiological role cannot, without further research, be excluded. The fact that little space is given to consideration of the ætiology of human rectal cancer is a measure of the paucity of research in this field.

None of the criticisms made here is more than minor, and they detract very little from the value of the contents of the book. The contributors and editors are to be congratulated on the clarity of style and presentation, on the high quality of most of the tables and illustrations, and on the remarkable degree of continuity. Moreover, the choice of paper and size of type are admirable. The book will be of value to a wide variety of qualified doctors whether primarily concerned with surgery or not. In addition, the figures for operative mortality and 5-yr. survival will provide a useful yardstick by which surgeons can measure their own prowess when using the same techniques and by which the value of new techniques can quickly be assessed. Francis J. C. Roe

[1] Mohs, F. E., *Chemosurgery in Cancer, Gangrene and Infections* (C. C. Thomas, Springfield, Illinois, 1956).

DEVELOPMENTS IN VETERINARY SCIENCE

Advances in Veterinary Science

Vol. 6. Edited by C. A. Brandly and E. L. Jungherr. Pp. xi+382. (New York: Academic Press, Inc.; London: Academic Press, Inc. (London), Ltd. 1960.) 12 dollars.

THIS volume, which follows the pattern of previous ones, covers seven main subjects. In the first chapter, screw-worm research and eradication are dealt with. Details are given of the biology of the screw fly, its geographical distribution and the means of rearing it under laboratory conditions. Chief interest in this section will probably be found in the discussion on the means of screw-worm eradication by releasing sterilized males in an infested area. The genetic basis for sterilization by irradiation is discussed in a concise but thorough manner. Insecticides in the form of wound dressings, sprays and dusts, including organo-phosphorus compounds, are discussed in the concluding chapters of this section.

In the second chapter foot-and-mouth disease and related vesicular diseases are dealt with in considerable detail. Although a number of the facts set out

here are already well known, they are very wisely reiterated in order to form a correct basis for the subsequent discussion. The distribution of foot-and-mouth virus types and sub-types, the ætiology, prevention, control and eradication as well as diagnosis are covered in some detail. At the present time, when the control of this disease throughout the world is receiving active attention, the information on types of vaccine and their preparation and application is of more than academic interest. Vesicular stomatitis and vesicular exanthema, virus diseases which show so many clinical signs similar to those of foot-and-mouth disease, are discussed from the same angle. While in Great Britain the absence of these two diseases makes the diagnosis of foot-and-mouth disease less complicated, the position in the United States is not so simple, and differentiation between the three viral diseases covered in the chapter is a practical necessity in those States in which all three could occur simultaneously.

To those primarily interested in animal husbandry, the chapter dealing with the effects of nutrition on microbial disease will have a special appeal. Any confusion of thought on what really constitutes disease, infection, nutrition and ecology is dispelled by the critical and clear definition of these terms with which the chapter begins. The authors summarize the effects of the various food constituents on the prevention of patent disease in the animal.

In summarizing their conclusions, they liken the balance between the host and micro-organism to a game of chess in which the result more commonly depends on a series of moves and counter-moves than on a single finalizing move, and point out that the micro-organism may in many cases overcome its host by the narrowest of margins.

The fourth chapter deals with anaplasmosis, covering historic development, distribution and incidence, and the evolution of epizootics in the United States. The epidemiology of the two main types of anaplasma and of organisms of similar type but doubtful nature, such as eperythrozoon, are given due attention. Transmission of the parasite by ticks, flies and mosquitoes and transmission *in utero* and accidentally by direct contact with infected animals are discussed. Investigations of the parasite, including the use of electron microscopy, problems of its reproduction, its cultivation, its properties and its pathogenesis (aided by the use of radioisotopes), occupy a considerable part of this chapter and will be found to be of fundamental importance to those interested in this protozoan blood parasite. Diagnosis by clinical and laboratory means, the pathology of the disease and its prophylaxis and therapy, with a note on the future for its control, complete a full and valuable description of this widespread disease of the blood.

The fifth chapter covers a great deal of ground without being unduly long. It deals with the comparative pathology of arthritis and embraces the many forms of arthritis in all classes of livestock, including birds. In the concluding remarks to this chapter, it is pointed out that the chemical composition of articular cartilage has not yet been studied on a comparative basis and that little is known of the character of normal synovial fluid in the various species. This observation must surely suggest lines for future research.

The following chapter deals with coccidioidomycosis. This fungal disease, which has been found to affect both animals and man, is described from the point of view of its history, its incidence, its classification (including its saprophytic and parasitic forms) and the conversion from one form to the other. With inhalation as the usual mode of infection of men and animals, the epidemiology of the disease makes an interesting study, and it is noted that certain races of humans and certain breeds of animals are more susceptible than others. Pathogenosis and pathology are discussed, and diagnosis, prognosis and treatment are dealt with. The public health significance of the disease renders an investigation of it all the more important.

The final chapter deals with canine distemper, a disease of which so much has been written. Here we find the more important considerations condensed into a valuable and all-embracing treatise. The question is posed: "Does distemper produce a long-lasting immunity because there is one serological type or, conversely, is there one serotype because the virus evokes a high-grade immunity?".

As in the case of previous volumes, this sixth volume contains a comprehensive list of references on each of the subjects discussed as well as author and subject indexes of considerable value to the reader. The high standard set by previous volumes is fully maintained and another addition of outstanding value to the teacher and research worker will find its way on to their bookshelves, not to lie there but to be consulted freely and profitably.

HAROLD BURROW

THE FATTY ACIDS

Fatty Acids

Their Chemistry, Properties, Production, and Uses. Part 1. Edited by K. S. Markley. Second completely revised and augmented edition. (Fats and Oils : a Series of Monographs on the Chemistry and Technology of Fats, Oils, and Related Subjects.) Pp. ix+714. (New York : Interscience Publishers, Inc. ; London : Interscience Publishers, Ltd., 1960.) 169*s*.

THE first edition of this work was published in 1947 as a single 668-page volume, written entirely by K. S. Markley. The material covered has now been expanded to such an extent that this second edition occupies four parts, each with its own index. Part 1 is concerned with the content of the first third of the 1947 edition and has chapters by Markley on the individual acids and isomerism (250 and 36 pages, respectively), by R. T. O'Connor on X-ray diffraction and polymorphism (94 pages) and spectral properties (120 pages), and by W. S. Singleton on the properties of the liquid state and of solutions (111 and 70 pages, respectively).

Markley's section on the acids has more than 600 references and covers most types of aliphatic carboxylic acid—natural and synthetic, short- and long-chain, saturated and unsaturated, and oxygenated. The acids are dealt with individually, the main points of discussion being occurrence, structure, preparation, applications and properties. The more important acids, industrially as well as academically—such as oleic acid and the naturally occurring acetylenic acids—are treated at some length, though the approach tends to be historical and somewhat classical rather than critical. The tables of physical constants and other properties are numerous and well presented. Unfortunately, the literature coverage does not appear exhaustive, though it is certainly extensive.

Those vital adjuncts of modern organic chemistry, the physical methods, are expertly and concisely introduced and the relevance and application of the main methods to the fatty acid field are well brought out. X-ray diffraction, ultra-violet, visible, infra-red and micro-wave spectroscopy, and nuclear magnetic resonance are each discussed. The estimation of *trans*-isomer content by infra-red spectroscopy and other similarly important applications are carefully documented though, again, I would have preferred a more critical appraisal of the present situation.

The properties dealt with under 'the liquid state' include heats of formation and combustion, density, molar volume, viscosity, surface tension and refractivity, many of the data being economically presented in tabular form. The final chapter surveys the properties of aqueous and non-aqueous solutions of the acids and their glycerides.

In summary, Part 1 of this completely rewritten and enlarged edition of *Fatty Acids* provides an unrivalled survey of the field covered. The complete work, which will consist of Parts 1, 2, 3 and 4, should prove a worthy successor to the first edition.

GEOFFREY EGLINTON

COTTON IN INDIA

Cotton in India

A Monograph, Vol. 2. By R. H. Dastur, R. D. Asana, K. Sawhney, S. M. Sikka, R. S. Vasudeva, Quadiruddin Khan, V. P. Rao and B. L. Sethi. Pp. viii + 339 (16 plates). Rs. 30; 45*s*.

Vol. 3. By C. Nanjundayya, R. L. N. Iyengar, W. R. Natu, M. B. Ghatge, K. S. Murti, C. B. Parikh, B. L. Sethi and D. N. Mahta. Pp. viii + 295 (20 plates). Rs. 30; 45*s*.

Vol. 4. By S. M. Sikka, Arjan Singh, Avtar Singh, P. D. Gadkari, R. Balasubrahmanyan, N. K. Iyengar, K. Sawhney, V. K. Bederker, G. B. Patel, P. S. Pandya, V. N. Paranjpe and N. S. Panigrahi. Pp. x + 378 (20 plates). Rs. 30; 45*s*.

(Bombay: Indian Central Cotton Committee, 1961.)

THE monograph *Cotton in India*, the publication of which has been sponsored by the Indian Central Cotton Committee, is now completed by the issue of Volumes 2, 3 and 4. Volume 1 has already been reviewed in *Nature*, **189**, 86 (1961).

The second volume deals with physiology, agronomy, diseases, insect and mite pests, and with seed multiplication and distribution. The third volume concerns itself with technology, ginning and pressing, marketing, consumption of cotton (seed and lint), legislative measures and exports and imports. The fourth and final volume treats of the special features of the six recognized cotton regions of India.

As the work covers every possible aspect of cotton in India, with a large number of contributors, it is difficult to discuss any points in detail. Practically all work done in India is mentioned and, though some can only be described as trivial, important findings occur sporadically throughout the work. The laborious and painstaking, though somewhat unfruitful, work of Dastur on applied physiology deserves special mention. He points out that no fundamental physiological research has so far been attempted as all attention has been concentrated on the physiological causes of bud and boll shedding, bad opening of bolls with immature seeds and lint, premature yellowing and reddening of leaves, low yields of seed cotton, etc. The important work on cotton physiology in Trinidad by Mason and his co-workers, Maskell and Phillis, receives only scant attention. In Egypt much fundamental work was done by Balls and his colleagues, much of which has some bearing on Indian problems. There is a casual reference to the *Cotton Plant in Egypt* published in 1912, but later work during 1927–47 is ignored. The subterranean environment, probably as important as the aerial one, does not seem to have been studied, and it is significant that 'roots' finds no place in the index.

In Volume 3, several pages in the chapter on technology are devoted to an exposition of statistics at the elementary text-book level. This inclusion would appear to be unnecessary. The section on small-scale spinning-tests covers less than a page and is inadequate. Although of great importance to India, the pioneer small-scale spinning-plant set up by Balls and Hancock in Egypt is not mentioned.

The index can only be described as amateurish and calamitously inferior. It is neither comprehensive nor usable, and is quite unworthy of the encyclopædic nature of the work. The virtues of the monograph, however, greatly outweigh the defects, which regrettably include an undue number of printer's errors. On the whole, it is a reasonably good attempt to put on record what is known about cotton in India and will undoubtedly find wide use as a standard work of reference.

S. C. HARLAND

SOME ASPECTS OF ASIATIC RUSSIA

Physical Geography of Asiatic Russia

By S. P. Suslov. Translated from the Russian by Noah D. Gershevsky. Edited by Joseph E. Williams. Pp. xiv + 594. (San Francisco and London: W. H. Freeman and Company, 1961.) 105*s*.

THIS book—a large quarto, printed on good glossy paper and containing well-reproduced text, maps and diagrams as well as photographs, although these as a rule are not so well reproduced—possesses all the potentialities of a good and informative book. Actually it suffers from many original and translation defects.

First and foremost the book purports to be the *Physical Geography of Asiatic Russia*, but this title does not accurately describe the contents of the book. Physical geography must clearly include geomorphology and structural geology. In these fields the book is very weak, whereas the sections on hydrography, soils, plants and animals are adequate and well presented. The names of plants and animals are translated into vernacular English and their Latin names are printed in brackets. Their habit and ecology are well described—the general impression being that the writer is a specialist in bio-geography, not physical geography. On the other hand, the geomorphological and geological sections are written in a casual and often inexpert manner, occupy a small proportion of the text, are often disposed throughout the text and are inadequately illustrated by geological maps and sections. This is particularly annoying since great progress has been made during the past hundred years in the study of the geology and geomorphology of the Asian part of Russia, as evidenced by the work of such authorities as Obruchev, Shatsky and Nalivkin.

Returning to the title, we read *Physical Geography of Asiatic Russia*. Here it must be pointed out that the frontiers of Asia with Europe follow the Urals watershed, the Ural River and the watershed of the Great Caucasus Chain. In the book the eastern Uralian region and the Transcaucasian region are excluded. This means that in the first part of this book, entitled "Western Siberia", which comprises the greater part of the River Ob', all the headwaters of its left tributaries and all the important mining and metallurgical zone of the Uralian region are excluded. The exclusion of Transcaucasia means also the exclusion of a very important part of the Alpine orogenic belt, stretching from the Carpathians to the Hindu Kush.

The first three parts of this book deal with Siberia, while the fourth and last part deals with central Asia. The territorial division is done in a very peculiar manner, which agrees neither with the leading geological nor with the geographical features. The first part, entitled "Western Siberia", embraces the western Siberian lowlands, Altai, Salair and the Kuznetsk basin. So far, so good. One would expect then that Part 2 would deal with central Siberia, but its title is "Eastern Siberia", and the title of its first chapter (Chapter 4) is also "Eastern Siberia", while the title of its Chapter 6 is "Central Siberia". For a century, the plateau region situated between the Rivers Yenisey and Lena has borne the name of "Siberian Platform", as it is characterized by a unity of geological-tectonic structure and a unity of morphological features. Now, according to the authors, the northern part of the "Siberian Platform" receives the name of "Eastern Siberia" and the southern part "Central Siberia". Under" Eastern Siberia" are also included Sayan, Tuva, Cis- and Trans-Baikal regions—mountainous regions most properly linked with Altai–Salair–Kuznetsk basin. Again, Part 3, entitled "Far East", is made to embrace Amour and Maritime Province, which is the true Far East, as well as Sea of Okhotsk coastal region and the Kamchatka-Kurile region, the two western provinces which, in spite of their situation, are not generally called the "Far East". Part 4 deals with central Asia, which is plausibly divided into semi-desert, desert and mountain regions.

The results of this peculiar regional planning are obvious. Geologically and geomorphologically Altai, Salair, Kusnetsk, Sayan and Tuva represent a definite structural-tectonic unit, which, in this book, is broken up into various disjointed chapters. The balance struck between the geological–morphological and other aspects is illustrated by a typical chapter on the Altai in which, out of 45 pages, 19 are devoted to bio-geography, 6 to geology and 8½ to morphology. In other chapters, such as Chapter 4, dealing with part of the central Siberian Platform, there is no section on geology. In Chapter 5, dealing with the Arctic, the most important geological structure of Taimyr is not described at all. The book has only one proper geological map (Kuznetsk basin) in addition to a tectonic map of the Transbaikal region and a few maps of Kamchatka-Kuriles.

The book would be greatly improved if it had an introductory chapter in which the main geological, tectonic and geomorphological features were discussed. Permafrost, a phenomenon typical of a very large part of Siberia, would be much more effectually discussed in an introductory chapter than within the confines of Chapter 4. Because of this chopping up of Siberia into unnatural regions, a very important geological feature has been missed out—namely, the Siberian shield, and its associated plateau lava region, one of the most extensive in the world—the so-called "Siberian Traps". Not only do the numerous basalt lava flows and sills determine the landscape of central Siberia, but also these rocks are often associated with valuable ore deposits and with the newly discovered kimberlite diamond bearing pipes. But diamonds are not mentioned in the general index of this book, neither are important tin deposits of the Sikhote-Alin Range, nor many ores of iron, lead, silver and gold, nor coal nor oil. All these are important ingredients of physical geography.

The translation has not been properly revised, and the number of glaring mistranslations or mistakes in the original text is large. Here are a few examples: p. 4, "Paleozoic sea" for "Paleogene sea"; p. 5, "Paleocene" for "Paleogene", "Neocene" for "Neogene"; p. 106, "Mount Shoria" for "Mountainous Shoria" (Gornaya Shoria); p. 115, "Sapropel" (organic mud) for "Sapropelic coal"; p. 115, "Stratified intrusive vein" (of basalt) for "sill" (of basalt); p. 188, "Tungus formation" for "Tunguska formation"; p. 208, "Mesozoic phase of Alpine folding" for "Kimmerian folding"; p. 233 "Peridite" for "peridotite"; p. 257, "Basalt deposits" for "intrusive basalt"; p. 269, "Tuva" (correct) in the text and "Tuvin" (wrong) in maps 8-V and 8-VI; and p. 289, "Caledonian = Lower Paleozoic" for "Caledonian = Upper Paleozoic".

The general impression is that this book was rapidly thrown together by a person or persons more interested in bio-geography than in geology and that the geological–morphological part of it is very poor and contains many original and translation errors.

S. I. Tomkeieff

INFINITE-DIMENSIONAL ALGEBRAS

Normed Rings

By M. A. Naimark. Translated from the First Russian edition by Leo F. Boron. Pp. xvi+560. (Groningen: P. Noordhoff, Ltd., 1959.) 45 D.fl.; 12 dollars, paper bound. 48.75 D.fl.; 13 dollars, cloth bound.

THE theory of finite-dimensional algebras, or hypercomplex number systems, as they used to be called, is one of long standing, going back to Hamilton's quaternions, but only in the past twenty years or so has there been created a general theory for the infinite-dimensional case. As in the case of vector spaces, one has to introduce some notion of continuity to obtain a useful theory; the algebraic operations are required to be continuous with respect to an appropriate topology, which, in the simplest cases, is defined by a norm, and we then have a normed algebra.

The most familiar normed algebra is that formed by the continuous functions on a closed interval of the real line, the algebraic operations being defined in the natural way and the norm being taken as the upper bound of the absolute value of the function. Another important algebra is formed by the set of all functions integrable over the whole real axis, multiplication being defined as convolution, that is:

$$(f*g)(x) = \int_{-\infty}^{\infty} f(y)g(x-y)dy$$

the norm being the integral of the absolute value of the function; this is a special case of the group algebra of a locally compact topological group. These algebras are commutative; important non-commutative algebras arise when we consider continuous linear operators in a Hilbert or Banach space.

Naimark's book is an admirable survey of the subject up to the appearance of the Russian edition in 1956. The 154 pages of the initial chapter could scarcely be bettered as a brief introduction to the basic ideas of functional analysis. The fundamental notions of normed algebras are then introduced; we next come to an account of Gelfand's theory of commutative algebras and their representation by means of continuous functions on a topological space formed by the maximal ideals of the algebra, and its extensions by Shilov and others. This is followed by a discussion of the representation of symmetric algebras by means of operators in Hilbert space and descriptions of some special types of algebras. The chapter on group algebras constitutes an excellent account of the theory of harmonic analysis on commutative locally compact groups; the non-commutative theory, which is still in a state of rapid development, is touched on lightly. The book concludes with a discussion of von Neumann's operator algebras in Hilbert space.

Throughout the book the author concentrates mainly on the analytic aspects and applications of the subject; a more algebraic point of view is to be found in Rickart's recent book on Banach algebras. The translation is well executed, with only occasional slight infelicities; it is a pity, however, that Mr. Boron has not followed the example of the German translation by using 'algebra' instead of 'ring', and so avoided a certain danger of confusion. F. Smithies

COMPUTING UP TO DATE

Modern Computing Methods

Department of Scientific and Industrial Research: National Physical Laboratory. Notes on Applied Science, No. 16. Second edition, completely revised. Pp. vi+170. (London: H.M.S.O., 1961.) 21*s*. net.

THIS second edition of the handbook prepared by staff of the Mathematics Division, National Physical Laboratory, is extremely welcome. A complete revision of the 1957 version has been made, revealing the considerable advances of the past few years, with particular emphasis on automatic computing, and also a greater appreciation of the purpose which the book fulfils. A hard cover in dark blue adds a becoming air of respectability and greater permanence.

The new edition opens with five chapters on linear equations and matrices, including a new one on error analysis, followed by chapters on zeros of polynomials, finite-difference methods, Chebyshev series, two on ordinary and two on partial differential equations, and one each on evaluation of limits, evaluation of integrals, and tabulation of mathematical functions. As before, there is a valuable bibliography consisting of carefully selected and annotated references.

There are many interesting changes of emphasis between the first and second editions. Relaxation methods are now given passing mention only, whereas nearly twelve pages are devoted to Chebyshev series. Graeffe's method and the Aitken–Bernoulli process have disappeared from the chapter on solving polynomial equations, and stress is placed on the ill-determination of the roots of high-order polynomials. The names of Givens and Householder now appear in connexion with the latent roots of matrices. It is perhaps in the chapters on partial differential equations that there is least evidence of progress.

Modern Computing Methods should not be regarded as a text-book, nor merely as a book of reference. It is a valuable supplement to any good book on numerical analysis, providing a critical selection and appraisal by experts of methods which have been put to the test. For this reason it must appeal both to teachers of numerical techniques and to practitioners. Not everyone will agree with the choice made, but after all experts must still be allowed to disagree. We should wish a great success to this second edition and hope that the hard cover does not betoken a life of more than three or four years, after which the third edition will be eagerly awaited.

R. A. Buckingham

MASER THEORY

Elements of Maser Theory

By Arthur A. Vuylsteke. Pp. xiii+362. (Princeton, N.J.: D. Van Nostrand Company, Inc.; London: D. Van Nostrand Company, Ltd., 1960.) 71*s*. 6*d*.

THE subject-matter of this book falls into two parts, each of which occupies roughly the same amount of space. The first is introductory in nature, and covers quantum theory, statistical mechanics, radiation and the interaction of radiation with matter, together with a treatment of the necessary mathematical techniques. The second part deals with the theory of two- and three-level masers, covers the ammonia maser and the paramagnetic solid maser in detail, and ends with a short discussion of noise theory in general and maser noise in particular. Mr. Vuylsteke writes with admirable clarity; but one is tempted to question the value of books of this type which attempt to do too much. The detailed study of masers is unlikely to be necessary for the student who might well find much of value in the first half of the book; the professional theoretician, on the other hand, might consider that this part merely adds to the bulk of what could have been a more modest monograph.

The section on masers proper is a most useful summary of the theory underlying microwave masers. Practical details are mentioned only when they are necessary for appreciation of the theory, or when they themselves have points of theoretical interest. The bibliography, as the author admits, is by no means exhaustive, and is complete enough only to support the text; it is a pity that this opportunity could not have been taken to provide the reader with a complete set of references. The style is lucid, and the author appreciates the value of explanatory interpolations to bring out essential points. Such is the rapidity of developments in this field that the optical maser now fashionable is not mentioned at all; but most of what need be said on microwave maser theory is to be found here. The book will prove invaluable as an introduction for those approaching the subject for the first time, and as a summary of the theory for those already familiar with the field.

J. H. Sanders

Nuclear Reactor Stability

By Dr. A. Hitchcock. (Nuclear Engineering Monographs, No. 11.) Pp. x+61. (London: Temple Press, Ltd., 1960.) 12*s*. 6*d*. net.

THE neutron multiplication in a reactor is affected by changes in temperature and other physical variables, which are in turn affected by changes in power. This circular situation can cause small changes in power-level to build up, so that the reactor becomes unstable. The possibility of unstable behaviour limits the available power output and must be either corrected by control action or, if possible, avoided by correct design. This monograph presents concisely, but with an admirably lucid and readable style, an introduction to the theory of reactor stability and the present methods used in establishing conditions for the stability of solid- and liquid-moderated power reactors, a theory which over the past few years has led, ahead of experimental confirmation, to important advances in power reactor control. The treatment, avoiding the use of specialized techniques and the jargon of control system analysis, assumes only an elementary knowledge of linear differential equations and Fourier series.

The first two chapters show how the physical mechanism of several kinds of instability may be related to the partial differential equations of reactor dynamics. In the third and fourth chapters the modal expansion method is applied to yield conditions for instability, first due to temperature effects and then due to the relatively slow effect of fission product (xenon) poisoning. The last two chapters deal with voidage instability in boiling reactors and the principles of spatial control.

Proceedings of the International Conference on Nuclear Structure

Kingston, Canada, August 29–September 3, 1960. Edited by D. A. Bromley and E. W. Vogt. Pp. 990. (Toronto: University of Toronto Press; Amsterdam: North-Holland Publishing Company, 1960.) 125*s*.

A FREQUENT criticism of conference proceedings is that they tend to present a heterogeneous collection of papers which might more suitably and more speedily have been published in the appropriate technical journals. This criticism does not apply to the volume under review. Here we have a well-edited handbook of contemporary ideas on nuclear structure. It leads in logical sequence from a consideration of the properties of nuclear forces and infinite nuclear matter to a series of contributions on the detailed properties of levels in actual nuclei. The book is interfused throughout with contributions on the various reaction mechanisms by which our knowledge of nuclear structure is obtained. It is excellently produced with good, clear type and is well illustrated with diagrams reproduced from the contributors' slides. This is particularly praiseworthy because this book was available to the delegates within five weeks of the closing date of the conference.

The editors have succeeded in effecting a pleasing compromise between the linguistic correctness of a submitted manuscript and the informality of expression in the conference hall. This happy blend has been much aided by the inclusion of verbatim reports of the lively discussion which followed the main contributions. This book has been made what a good conference report should be. It provides those who were there with an accurate record of what was said, while at the same time it preserves something of the atmosphere of the conference hall for their less fortunate colleagues who were unable to be present.

D. L. ALLAN

Progress in Dielectrics

Vol. 3. Edited by Dr. J. B. Birks and Prof. J. Hart. Pp. vii+292. (London: Heywood and Co., Ltd., 1961.) 63*s*. net.

THE third volume of this series is lively and well written. Three of the articles are concerned with electrical properties and molecular structure. R. H. Cole's review "Theories of dielectric relaxation and polarization" is helpful by its lucidity even when it covers old ground, and it contains some illuminating remarks, for example, on the dynamics of the Onsager equation. J. B. Hasted's article "The dielectric properties of water" (and ice) fills a long-felt want. This subject might deserve a book, particularly since it is relevant to biology. Hasted also gives a useful review of microwave techniques. R. J. Meakins systematically reviews new work on long-chain crystals, hydrogen-bonded compounds, occlusion compounds, and other types of solids the structure of which implies distinctive features of dielectric relaxation.

R. Stratton's review of theories of electrical breakdown is well presented, but this subject is perhaps being reviewed too frequently. The article by D. G. Kiely, "Dielectric waveguides and aerials", presents a mathematically difficult subject in a manner comprehensible to the experimentalist. The readable article by C. W. Hamilton on "Recent developments in cable insulation in the United States" is interesting in that it illustrates the gap which still exists between the questions asked by the engineer and the answers provided by the physicist and chemist.

V. DANIEL

Electrical Activity of Single Cells

Edited by Yasuji Katsuki. Pp. viii+312. (Tokyo: Igaku Shoin, Ltd., 1960.) n.p.

THIS book consists of 18 separate articles by different workers in the field of electrophysiology. Their common feature, given by the editor as reason for grouping these articles together, is that they all represent work done in Japan with the intracellular capillary micro-electrode. The subjects of investigation include sensory receptors, peripheral and central nerve cells, the neuromuscular junction and cardiac muscle. Each article has the strictly limited range of interest of a paper in a scientific journal. Furthermore, apparently because the numerous authors were intent on presenting as much of their experimental results as space allowed, very little attention is given to describing the method or to justifying its application in different experimental situations. This is particularly unfortunate here, as it is the method which is the unifying feature of the book. A welcome exception to this is an article by Takeuchi and Takeuchi, who give a careful account of the application of the voltage-clamp technique to the localized response of the neuromuscular junction.

Much of the material in this book has already been published in readily accessible journals (in English), and it is clear that the remainder will soon follow. The book does not therefore fulfil any general need.

P. FATT

BIOSYNTHESIS OF PORPHYRINS AND CHLOROPHYLLS*

By Dr. K. D. GIBSON, MARGARET MATTHEW, Prof. A. NEUBERGER, F.R.S., and G. H. TAIT
Department of Chemical Pathology, St. Mary's Hospital Medical School, London, W.2

IT is now generally accepted that all the carbon and nitrogen atoms of the hæm of hæmoglobin, myoglobin, catalase, peroxidase and the various cytochromes, and probably also those of the dihydroporphyrin of the chlorophyll of green plants and algae and those of the tetrahydroporphyrin of bacteriochlorophyll are derived from the one nitrogen atom and the five carbon atoms of δ-aminolævulic acid. The enzyme which is responsible for the formation of this acid, δ-aminolævulic synthetase, has been found in a great variety of cells and must be presumed to be present in all cells containing either hæm proteins or chlorophylls. This synthetase catalyses the condensation of glycine with succinyl-coenzyme A to give δ-aminolævulic acid, carbon dioxide and free coenzyme A. The enzyme has not been prepared in a high state of purity, but so far it appears that the only co-factor required for this reaction is pyridoxal phosphate.

The importance of this substance in the synthesis of δ-aminolævulic acid was indicated in the work of Lascelles[1] on porphyrin synthesis in *Tetrahymena vorax* and that of Schulman and Richert[2] on hæm synthesis in bird erythrocytes. The participation of pyridoxal phosphate in the enzymic reaction was later directly demonstrated independently by two groups of workers[3,4]. On the basis of the work of Braunstein and Snell, it seems reasonable to assume that pyridoxal phosphate (combined with a specific enzyme protein, possibly through its phosphate group) can react with the amino group of glycine to form an aldimine or Schiff's base, and this then reacts with succinyl-coenzyme A to form a new C—C bond. This reaction has been discussed in detail by Shemin *et al.*[5] and by one of us[6]; moreover, mechanisms of reactions of this type have recently been considered by Perault *et al.*[7], who have applied the molecular orbital method (L.C.A.O. method) to this problem. These workers have confirmed the belief that in the anionic form of the glycine–pyridoxal phosphate complex the carbon atom which is derived from the α-carbon atom of the amino-acid has a high electron density, and thus will react with the electrophilic carbonyl carbon atom of succinyl-coenzyme A.

Inhibition of δ-Aminolævulic Synthetase by Aminomalonate

The requirement for pyridoxal phosphate in the enzymic formation of δ-aminolævulic acid fully explains the inhibitory effects of substances like cysteine, penicillamine or cyanide, which are known to react with the aldehydic group of the coenzyme and thus prevent the formation of the aldimine. We have recently found that the formation of δ-aminolævulic acid can also be inhibited by aminomalonate. The effect of this substance is very marked, even at a low concentration. Kinetic investigation showed that the inhibition is of the competitive type with respect to glycine, and so far it appears that aminomalonate has no marked effect on other enzymes requiring pyridoxal phosphate. Monoethyl aminomalonate is a somewhat weaker inhibitor than aminomalonate, and the diethyl ester has only a relatively slight effect which is of the same order of magnitude as shown by many other amino-acids such as threonine, α-alanine or β-alanine. While kinetic investigations with crude enzyme preparations cannot be considered to give results of exact quantitative significance, the following values give some information of the inhibitory power of aminomalonate: K_m for glycine, $1{\cdot}4 \times 10^{-4}$ *M*; K_i for aminomalonate, $2{\cdot}2 \times 10^{-5}$ *M*; K_i for monoethyl aminomalonate, $3{\cdot}1 \times 10^{-5}$ *M*. It seems likely that aminomalonate combines with the pyridoxal phosphate – enzyme complex, the amino group forming an aldimine with the aldehyde group of pyridoxal. One or two carboxyl groups probably combine with part of the enzyme protein and the resulting complex which cannot react further with succinyl–coenzyme A may be sterically more favoured than the corresponding complex with glycine, which is the intermediate in the enzymic synthesis of δ-aminolævulic acid.

Transamination of δ-Aminolævulic Acid

Shemin and Russell[8] found that with rats given δ-aminolævulic acid labelled with carbon-14 in the carbon atom bearing the amino group, the isotopic carbon appeared in substantial amounts in respiratory carbon dioxide, urinary formate and in the ureido groups of uric acid. They proposed that δ-aminolævulic acid was metabolized by a cyclic pathway, the succinate–glycine cycle, involving the formation of a one-carbon intermediate derived from the δ-carbon of δ-aminolævulic acid. The general metabolic importance of this pathway cannot be assessed at present and the enzymic steps involved have not as yet been properly defined with the exception of the first reaction, the conversion of δ-aminolævulic acid to γ,δ-dioxovaleric acid. A specific transaminase catalysing this reaction has been found in mammalian tissues and in *Corynebacterium diphtheriae*[9,10]; we have further examined this enzymic reaction as it might provide a possible control mechanism for the synthesis of hæm and chlorophyll. The earlier workers investigating this reaction have incubated δ-aminolævulic acid and α-oxoglutarate and measured the glutamic acid produced. However, using extracts from *Rhodopseudomonas spheroides*, we have found that the equilibrium appears to be in favour of the production of δ-aminolævulic acid, and we have therefore incubated γ,β-dioxovaleric acid (supplied by Dr. A. T. Carpenter) with the amino-acid, and estimated δ-aminolævulic acid, for which a sensitive method is available. Moreover, we have found that L-alanine and β-alanine are from five to seven times more effective than glutamic acid in this reaction. Other amino-acids tested showed very little activity. The *p*H optimum is 7·0–7·2, and the reaction is strongly inhibited by pyruvic acid and methylglyoxal, whereas

* Substance of two lectures given by Prof. A. Neuberger at University College, London, on May 8 and 11, 1961.

oxoglutarate has little effect. So far no evidence has been obtained which would indicate that the transaminase has any controlling effect on the synthesis of porphyrin or chlorophyll in *Rps. spheroides*.

Role of Biotin in the Synthesis of Porphyrin and Bacteriochlorophyll

In an earlier account by one of us[6], the effect of biotin in these reactions was considered, and the possibility was discussed that it acts by catalysing the decarboxylation of α-amino-β-oxoadipic acid present as an intermediate in the pyridoxal phosphate – δ-aminolævulic acid synthetase complex. The evidence in favour of such an assumption was the finding (since confirmed by Lascelles[11]) that δ-aminolævulic acid synthetase activity in extracts of organisms grown in media containing very low concentrations of biotin was apparently specifically reduced if compared with those of δ-aminolævulic acid dehydrase, threonine dehydrogenase, β-hydroxybutyric dehydrogenase or alanine – δ-aminolævulic acid transaminase. The finding, however, that illumination of biotin-deficient organisms with addition of biotin to the medium produced growth and synthesis of porphyrin and bacteriocholorophyll without any increase in the synthetase activity as measured in extracts indicates that any effect of biotin is not exercised directly through a control of the level of δ-aminolævulic acid synthetase activity. Moreover, it was found that other conditions which are unfavourable to formation of bacteriochlorophyll by *Rps. spheroides*, such as a deficiency in nicotinic acid or thiamine, or replacement of malate in the growth medium by acetate, are associated with a depression of δ-aminolævulic acid synthetase activity. It therefore appears that the effect of biotin on the level of this enzyme is unspecific, and that the depression of the synthesis of porphyrin and bacteriochlorophyll by biotin deficiency is caused by some other mechanism, possibly through a reduction in the concentration of pyridoxal phosphate in the cells.

It was also reported earlier[6] that both a commercial preparation of avidin and preparations made in our laboratory by Dr. N. M. Green, containing 2·5 and 4·9 units of avidin/mgm. protein inhibited δ-aminolævulic acid synthetase activity in crude extracts of *Rps. spheroides*. This inhibition appeared to be specific; but when this 'avidin' was pre-incubated with biotin, inhibition was still observed. This seemed surprising, as other biotin-containing enzymes are not inhibited by avidin which has been incubated with biotin before being added to the enzyme under investigation. However, when Dr. Green achieved a further purification of the avidin by column chromatography and made a preparation available to us which had an activity of 9·6 units/mgm., and an estimated purity of 80 per cent, no inhibition of δ-aminolævulic and synthetase activity was obtained. No inhibition was observed with other egg-white proteins such as ovalbumin, lysozyme and ovomucoid, and it must be concluded that some protein not yet identified and which contaminates crude avidin, has a specific inhibitory effect on δ-aminolævulic acid synthetase.

Chemical Conversion of Porphobilinogen to Uroporphyrins

The next step in porphyrin biosynthesis is the conversion of δ-aminolævulic acid to porphobilinogen. This reaction is catalysed by the enzyme δ-aminolævulic acid dehydrase and is fully discussed in a recent review of Bogorad[12]. Porphobilinogen is then transformed to uroporphyrinogen by enzymes to be discussed later, but first it is worth while to consider briefly the non-enzymic reaction which occurs fairly readily under relatively mild conditions. It has been known for some time[13] that on heating porphobilinogen in acid solution, there is obtained a mixture of uroporphyrins in good overall yield and that this mixture contains an appreciable amount of uroporphyrin III. These findings were confirmed by Westall, who isolated porphobilinogen in pure form[14], and by Cookson and Rimington[15], who determined its structure and showed it to be an intermediate in porphyrin biosynthesis. Recently, Mauzerall[16] improved the analysis of individual isomers by decarboxylating the uroporphyrin and estimating the coproporphyrins after chromatographic separation by a modification of the method of Falk *et al.*[17]. In this way it was possible to determine the amounts of isomers I and II and the combined amounts of III and IV in a mixture. Mauzerall was thus able to study the formation of uroporphyrin semi-quantitatively and to determine the distribution of isomers, although the sensitivity of the method is still not very high and the fact that isomers III and IV cannot be separated makes interpretation somewhat difficult. The results obtained by Mauzerall and the earlier workers can be summarized as follows:

(1) The products of the reaction are not uroporphyrins, but uroporphyrinogens, that is, hexahydroporphyrins in which the pyrrole rings are linked through methylene bridges.

(2) If porphobilinogen or any of the four uroporphyrinogen isomers is heated in acid solution, the distribution of the resulting uroporphyrinogen isomers is as follows: 3/4 III + IV, 1/8 I and 1/8 II. In neutral or alkaline solution, no isomer II is formed from porphobilinogen and about half the total porphyrinogen was found to be isomer I.

(3) If the isomerization of uroporphyrinogen in acid is carried out in the presence of [^{14}C]formaldehyde, radioactivity is found in the final mixture of uroporphyrinogens; however, no such incorporation of formaldehyde takes place when uroporphyrins are heated in acid or when uroporphyrinogens are heated at neutral or alkaline *p*H. Radioactivity is found in uroporphyrinogens when these are formed by heating solutions of porphobilinogens in the presence of [^{14}C]formaldehyde and this occurs at acid, neutral and alkaline *p*H values.

(4) In the isomerization of uroporphyrinogen in acid solution and in the formation of uroporphyrinogen from porphobilinogen in acid or neutral solution, yields of up to 80–87 per cent of total porphyrinogen are obtained. Although some material behaving like dipyrrylmethanes was formed, no evidence was obtained for the presence of higher linear polypyrrylmethanes, and it must be assumed that once a linear tetrapyrrylmethane with one aminomethyl or hydroxymethyl group is formed, cyclization to the macrocycle, uroporphyrinogen, is very fast.

Enzymic Conversion of Porphobilinogen to Uroporphyrinogen

The enzymic conversion of porphobilinogen to uroporphyrinogen has been studied in extracts of *Chlorella*, spinach leaf and wheat germ, and in fowl erythrocytes, by Bogorad, Mauzerall, Granick and Rimington (for review see Bogorad[12]) and more recently by Lockwood and Benson[18], and in *Rps.*

spheroides by Heath and Hoare[19]. The main findings were as follows: the product of the reaction catalysed by the crude enzyme system is uroporphyrinogen III and not uroporphyrin III. When the crude enzyme system is heated to 55° or 60° (depending on *p*H) the product is not uroporphyrinogen III, but isomer I. From the heated extracts an apparently single enzyme called porphobilinogen deaminase can be obtained which can be further purified and which converts porphobilinogen quantitatively into uroporphyrinogen I. The second and more heat-labile enzyme, called uroporphyrinogen isomerase, has no detectable action on uroporphyrinogen I or on porphobilinogen alone. Its substrate is either a linear tetrapyrrole or, what seems less likely, a dipyrrolic or tripyrrolic substance which is converted by the isomerase to uroporphyrinogen III through further condensation with one or two porphobilinogen molecules.

Although, as pointed out above, isomer analysis of porphyrins is neither so sensitive nor so specific as might be desired, it appears from evidence at present available that enzymic condensation does not give rise to uroporphyrinogens II and IV. It also appears that formation of uroporphyrinogen by the two enzymes is quantitatively paralleled by consumption of porphobilinogen. Moreover, neither the intact nor the heat-altered enzyme preparation incorporates radioactive formaldehyde into porphyrin. This finding is in accordance with the observations of Bogorad[20] and of Carpenter and Scott[21,22], that opsopyrroledicarboxylic acid and isoporphobilinogen are not substrates for the deaminase or isomerase; in fact, the two substances act as competitive inhibitors.

Many suggestions have been put forward to explain both the chemical and enzymic condensation of porphobilinogen to uroporphyrinogen and particularly the proportions of isomers formed. Most of the schemes are not readily compatible with the experimental findings; particularly those obtained in the enzymic reaction. Porphobilinogen deaminase might perhaps best be visualized as catalysing the addition of a porphobilinogen molecule to the free α-position of another porphobilinogen molecule or a dipyrrole or tripyrrole of the structures *PA*.*PA*—$CH_2NH_3^+$, *PA*.*PA*.*PA*—$CH_2NH_3^+$ (*A* stands for acetyl and *P* for propionyl side-chain). It is postulated that the action of this enzyme stops at the linear tetramer stage and that the deaminase is highly specific with regard to the nature of the β-substituents, both in the electrophilic and the nucleophilic partner of each step of the reaction. Alternatively, the condensation of the four porphobilinogen units on the enzyme surface may be simultaneous. In the absence of the isomerase the linear tetramer will cyclize spontaneously to give uroporphyrinogen I. The findings of Mauzerall[18] have clearly shown that at the tetrameric stage cyclization is greatly favoured over further linear addition, and this author has also adduced convincing reasons for such preference.

In an important paper, Mathewson and Corwin[23] suggested that the substrate of the isomerase is the linear tetramer and that this enzyme catalyses an attack by the $CH_2NH_3^+$ group, not on the free α-position of the pyrrole at the other end of the tetramer chain, but on the 'occupied' α-position of that pyrrole. The cyclic structure thus produced will then become re-arranged to give uroporphyrinogen III. Mathewson and Corwin have also proposed that three of the rings in the linear tetramer are in the pyrrolenine form, thus giving even greater flexibility to the structure. In the cell, co-operation between the two enzymes is almost perfect and thus only minute traces of uroporphyrin I are normally formed. In congenital porphyria this co-operation breaks down, with the result that the linear tetramer accumulates and this spontaneously changes into uroporphyrinogen I.

The mechanisms operating in the non-enzymic formation of uroporphyrinogens from porphobilinogen are more obscure than those involved in the enzymic reaction. The scheme proposed by Mauzerall[18] explains the incorporation of formaldehyde and the isomer ratios, but the suggested mechanism of Mathewson and Corwin, though satisfactory for explaining the enzymic condensation, does not readily accommodate the exchange with formaldehyde so convincingly demonstrated by Mauzerall.

Conversion of Uroporphyrinogen III to Protoporphyrin

The decarboxylation of uroporphyrinogen III to coproporphyrinogen is a straightforward reaction and requires little comment in this discussion. The next step, however, is more complex and has been investigated recently.

Sano and Granick[24] have described an enzyme which is present in a great variety of tissues, but is found in particularly high concentration in the liver, bone marrow and small intestine, and which converts coproporphyrinogen III to protoporphyrin IX. This enzyme, which has been purified approximately twenty times, is localized primarily in the mitochondria. The enzyme responsible for the condensation of succinyl coenzyme A and glycine to give δ-aminolævulic acid is also localized in particulate structures of the cell, whereas δ-aminolævulic acid dehydrase is present in the cytoplasm. It would therefore appear that δ-aminolævulic acid is synthesized in mitochondria, then leaks out into the cytoplasm, to be converted ultimately into coproporphyrinogen, which migrates back again into the mitochondria, to be transformed into protoporphyrin. Sano and Granick suggest that this localization might permit a control of porphyrin synthesis by the cell through permeability factors.

The enzyme which acts on coproporphyrinogen removes two hydrogen atoms and a carboxyl group from each of the two propionic acid side-chains in rings *A* and *B*, and at the same time porphyrinogen is converted to porphyrin. The latter reaction occurs fairly readily in the presence of oxygen and is not necessarily catalysed by an enzyme. The first two reactions, however, appear to be part of a concerted mechanism as the appropriate diacrylic acid is not an intermediate. Oxygen acts in this somewhat impure system as a hydrogen acceptor and cannot be replaced by any other co-factor tested. This is of considerable general importance, since it might indicate that oxygen was present in the atmosphere of the Earth at a time in evolution when protoporphyrin first appeared.

Biosynthesis of Chlorophyll and Bacteriochlorophyll

The work of Granick[25] has produced strong presumptive evidence that chlorophyll and bacteriochlorophyll are synthesized by green plants and

certain bacteria from protoporphyrin. Although the enzymic steps involved cannot as yet be clearly defined, Granick has proposed, mainly on the basis of work with mutants of *Chlorella*, that the following pathway operates:

protoporphyrin → magnesium protoporphyrin or protoporphyrin monomethyl ester → magnesium protoporphyrin monomethyl ester → → → magnesium vinyl phæoporphyrin a_5 → magnesium vinyl phæoporphyrin a_5 phytyl ester → chlorophyll *a* → bacteriochlorophyll

Although this is a rational scheme, it does not necessarily follow that compounds which accumulate in mutants, in which the enzyme for a particular step in a biosynthetic pathway is absent, are true intermediates. It is now known, for example, that intermediates in hæm synthesis are porphyrinogens and not uro- and coproporphyrins, although these latter substances may be accumulated or excreted under certain conditions. In a similar manner, protoporphyrinogen or one of its derivatives might be a precursor of chlorophyll.

We have recently been able to define at least one step in Granick's pathway more clearly. In work aimed at elucidating the effect of biotin, it was observed that ethionine at a concentration of about 10^{-4} *M* inhibited bacteriochlorophyll synthesis by *Rps. spheroides* almost completely, and at the same time produced a markedly increased excretion of copro- and uro-porphyrin into the medium in which the organism was illuminated. At that concentration very little inhibition of protein synthesis or growth occurred. Many other substances, such as methionine, other amino-acids, keto-acids and related compounds, amino-acid antagonists and other inhibitors of protein or nucleic acid synthesis, were tested to see whether they produced similar effects. The results were negative with the exception of threonine, the addition of which to the medium at a concentration of 10^{-3} *M* inhibited bacteriochlorophyll synthesis and produced a considerable increase in porphyrin excretion. The effects of ethionine and of threonine were reversed by methionine and by homocysteine, but not by cysteine or serine. The effect of threonine was also reversed by homoserine. This suggested that ethionine interfered either with the synthesis of methionine or its utilization and further that methionine had a specific function in the synthesis of chlorophyll from protoporphyrin involving the methyl-group donating capacity of this amino-acid. The effect of threonine was baffling at first, until it was realized that methionine and threonine share a biosynthetic pathway leading from aspartic acid through aspartyl β-phosphate and aspartyl β-semialdehyde to homoserine. The latter can be converted either to homocystoine and methionine or to threonine. Thus threonine could inhibit methionine synthesis from aspartic acid by a feed-back mechanism involving either repression or inhibition of one of the enzymes catalysing the various steps leading to homoserine. (Recent results have shown that in *Rps. spheroides* threonine is a competitive inhibitor of homoserine dehydrogenase, although it does not repress the formation of this or any of the other enzymes involved in homoserine synthesis.)

The most likely step in the biosynthesis of chlorophyll involving the methyl group of methionine is the methylation of protoporphyrin or of its magnesium complex to give magnesium protoporphyrin monomethyl ester. Organisms were illuminated in a suitable medium to which methionine labelled with carbon-14 in the methyl group was added. At the end of the experiment, bacteriocholorophyll was isolated, purified and counted. The chlorophyll had high radioactivity, which was shown on degradation to be entirely confined to the methyl ester group (Table 1). With appropriate amounts of [^{14}C]methionine the dilution was only about three times, and this indicated that the methyl group of methionine was almost certainly the immediate and specific precursor of the porphyrin ester carbon atom. The less specific utilization of formate for the methyl ester group of chlorophyll had already been shown some time earlier[26]. More recently two of us, using chromatophore preparations of *Rps. spheroides*, have been able to demonstrate directly the methylation of magnesium protoporphyrin to its methyl ester by *S*-adenosylmethionine[27].

Table 1. Labelling of Bacteriochlorophyll synthesized in the Presence of [Methyl-^{14}C]Methionine

Rps. spheroides were illuminated at 2 mgm. dry wt./ml. in Mixture I of Lascelles (ref. 31) for 18 hr. at 32-34°. [Methyl-^{14}C]methionine (10^{-4} *M*) was also present. The bacteriochlorophyll was extracted and purified. A portion of it was degraded in methanolic potassium hydroxide. The methanol, bacteriochlorin and phytol were isolated and estimated for radioactivity by liquid scintillation

Fraction	Specific radioactivity (counts/min./μmole)
Bacteriochlorophyll	9,800
Methanol	9,900
Phytol	64
Bacteriochlorin	6

Control of Synthesis of Bacteriochlorophyll

Micro-organisms such as *Rps. spheroides* or *Rhodospirillum rubrum* can grow either anaerobically in the light or aerobically in the dark, using as source of energy a number of aliphatic acids or alcohols. Elsden and Ormerod[28] showed that *R. rubrum*, under aerobic conditions in the dark, metabolized all substrates by the tricarboxylic acid cycle. Anaerobically in the light, acetate and butyrate are metabolized by this cycle, but propionate, malate and succinate are utilized by another pathway which is not inhibited by fluoroacetate. The question therefore arises how the type of metabolism is regulated. Following van Niel's pioneer work, this problem was first systematically investigated by Cohen-Bazire, Sistrom and Stanier[29]. They showed that organisms grown aerobically in the dark contained little pigment, whereas those grown anaerobically in the light contained relatively large amounts of bacteriochlorophyll; that transference of organisms from the dark to light resulted, after a lag phase, in synthesis of pigment; that exposure to oxygen of organisms growing in the light inhibited synthesis of pigment immediately, that is, without a lag phase, and removal of oxygen resulted in immediate resumption of synthesis of pigment and that increasing light intensity beyond a certain value had the same effect as an increase of oxygen tension. On the basis of these results, they suggested that the synthesis of both bacteriochlorophyll and carotenoids is controlled by the state of oxidation or reduction of a carrier of the electron-transport chain. They did not specify the identity of the substance postulated or the mechanism by which this control operates.

Lascelles[11,30] examined changes in the activities of various enzymes involved in porphyrin synthesis in extracts from cells grown under conditions under which the rate of bacteriochlorophyll synthesis is either high or low. When synthesis of pigment was inhibited or when levels of pigment were low, the

activities of δ-aminolævulic acid synthetase and dehydrase were also reduced, and synthesis of these enzymes was apparently inhibited. She also found that the transference of organisms from highly aerobic conditions in the dark to conditions of low oxygen tension in the dark, or to anaerobic conditions in the light, not only caused pigment synthesis but also resulted in an increase in the activities of δ-aminolævulic acid synthetase and dehydrase. This adaptation could be inhibited by inhibitors of protein synthesis. Lascelles[11] observed that hæmin represses formation of the synthetase and dehydrase in whole cells, and there was some evidence that formation of these two enzymes could be regulated by the concentration of iron porphyrins present in the cells. We have found that hæmin, in addition to its repressive effect on the formation of these enzymes, inhibits the synthetase activity in cell extracts and that protoporphyrin inhibits the dehydrase activity. Accumulation of hæmin and of protoporphyrin under certain conditions of growth may thus reduce synthesis of pigment both by repression of enzyme formation and by inhibition of the activities of enzymes already present in the cells.

There is, however, good evidence that levels of porphyrins and hæm are not the only factors controlling synthesis of pigment. The experiments on the effects of ethionine and threonine demonstrate the importance of the methylation step; and the excessive amounts of porphyrins excreted in the cells in the presence of moderate quantities of ethionine and threonine in the medium suggest that porphyrin formation is normally controlled by a substance *X*, possibly a phæoporphyrin derivative, which occurs after magnesium protoporphyrin in the biosynthetic pathway of bacteriochlorophyll. If this substance is not synthesized, for example, owing to inhibition of the methylation of magnesium protoporphyrin or to inhibition of methionine formation, porphyrin synthesis 'runs wild' and excessive amounts are formed which are released into the medium. In addition to these direct mechanisms of control, it appears that in general the rates of formation of chlorophyll and of carotenoids are closely parallel, and we know little about the factors regulating the formation of the latter. Similarly, phytol is a component of the bacteriochlorophyll molecule and its mode of synthesis is still obscure, although it would appear likely that it is derived from a carotenoid or a carotenoid precursor. Mechanisms which increase or depress terpene synthesis might therefore also control synthesis of bacteriochlorophyll through the availability of the phytol side-chain.

[1] Lascelles, J., *Biochem. J.*, **66**, 65 (1957).
[2] Schulman, M. P., and Richert, D. A., *J. Biol. Chem.*, **226**, 181 (1957).
[3] Kikuchi, G., Kumar, A., Talmage, P., and Shemin, D., *J. Biol. Chem.*, **233**, 1214 (1958).
[4] Gibson, K. D., Laver, W. G., and Neuberger, A., *Biochem. J.*, **70**, 71 (1958).
[5] Kikuchi, G., Kumar, A., and Shemin, D., *Fed. Proc.*, **18**, 259 (1959).
[6] Neuberger, A., *Biochem. J.*, **78**, 1 (1961).
[7] Perault, A.-M., Pullman, B., and Valdemoro, G., *Biochim. Biophys. Acta*, **46**, 555 (1961).
[8] Shemin, D., and Russell, C. S., *J. Amer. Chem. Soc.*, **75**, 4873 (1953).
[9] Kowalski, E., Dancewicz, A. M., and Szot, Z., *Bull. Acad. Polon. Sci.*, II, **5**, 223 (1957).
[10] Bagdasarian, M., *Nature*, **181**, 1399 (1958).
[11] Lascelles, J., *J. Gen. Microbiol.*, **23**, 487, 499 (1960).
[12] Bogorad, L., in *Comparative Biochemistry of Photoreactive Systems*, edit. by Allen, M. B., 227 (Academic Press, Inc., New York, 1960).
[13] Waldenström, J., and Vahlquist, B., *Hoppe-Seyl. Z.*, **260**, 189 (1939).
[14] Westall, R. G., *Nature*, **170**, 614 (1952).
[15] Cookson, G. H., and Rimington, C., *Biochem. J.*, **57**, 476 (1954).
[16] Mauzerall, D., *J. Amer. Chem. Soc.*, **82**, 2601, 2605 (1960).
[17] Falk, J. E., Dresel, E. I. B., Benson, A., and Knight, B. C., *Biochem. J.*, **63**, 87 (1956).
[18] Lockwood, W. H., and Benson, A., *Biochem. J.*, **75**, 372 (1960).
[19] Heath, H., and Hoare, D. S., *Biochem. J.*, **72**, 14 (1959).
[20] Bogorad, L., *Plant Physiol.*, **32**, xli (1957).
[21] Carpenter, A. T., and Scott, J. J., *Biochem. J.*, **71**, 325 (1959).
[22] Carpenter, A. T., and Scott, J. J., *Biochim. Biophys. Acta* (in the press).
[23] Mathewson, J. H., and Corwin, A. H., *J. Amer. Chem. Soc.*, **83**, 135 (1961).
[24] Sano, S., and Granick, S., *J. Biol. Chem*, **236**, 1173 (1961).
[25] Granick, S., *Harvey Lectures*, **44**, 220 (1948–49); *J. Biol. Chem.*, **236**, 1168 (1961).
[26] Green, M., Altman, K. I., Richmond, J. E., and Salomon, K., *Nature*, **179**, 375 (1957).
[27] Tait, G. H., and Gibson, K. D., *Biochim. Biophys. Acta*, **52**, 614 (1961).
[28] Elsden, S. R., and Ormerod, J. G., *Biochem. J.*, **63**, 691 (1956).
[29] Cohen-Bazire, G., Sistrom, W. R., and Stanier, R. Y., *J. Cell. Comp. Physiol.*, **49**, 25 (1957).
[30] Lascelles, J., *Biochem. J.*, **72**, 508 (1959).
[31] Lascelles, J., *Biochem. J.*, **62**, 78 (1956).

ANIMAL BEHAVIOUR

A SYMPOSIUM on animal behaviour was held on September 4 by Section J (Psychology) of the British Association for the Advancement of Science, during the recent meeting at Norwich. As was pointed out by the chairman, Mr. D. E. Broadbent (Medical Research Council Applied Psychology Research Unit, Cambridge), the Section has usually considered primarily human behaviour; but in this it is ceasing to be in step with university departments of psychology, which are devoting more effort than they once did to research on animals. Animal behaviour is a subject of great interest in itself. It also has relevance to human beings, because it reveals simple general principles about behaviour that may also apply to men, and it makes possible experiments on causal factors in behaviour which cannot, for moral and practical reasons, be performed on men. Thus the influence on behaviour of rewards, of the structure of the brain, of restricted infantile experience, and of drugs and instincts, can be studied in animals, and papers on each of these topics were included in the symposium.

Mr. H. M. B. Hurwitz and Dr. J. R. Millenson (Birkbeck College, University of London), proceeded to discuss and demonstrate the control of behaviour by reward. Their emphasis was on the possibility of building up valid empirical statements about observed relationships without appeal to theoretical concepts. Thus, if a response R was followed by a reinforcing agent S^r, such as food, the frequency of R would increase. If S^r ceased to follow R, the latter would decrease in frequency. Such statements could be made without any discussion of events within the animal. In a similar way, but with more complex experimental manipulations, behaviour such as problem solving or delayed reaction could be studied.

The authors then demonstrated a pigeon and a rat the behaviour of which had been shaped in a complicated fashion. The pigeon would look at three keys, peck the middle one, and observe a coloured light on

that key which was sometimes red and sometimes green. One of the other two keys was red and one green, and the bird would then peck the key which was the same colour as the middle key. This sequence occurred continuously at a rate rather faster than most humans could make the comparison. The method of training had been, first, to teach the bird that food would appear in a certain place when a light was on, then to teach that pecking either of the two end keys would produce the food; this training was followed by withholding the food unless a peck at the centre key was made before the peck at the end key, and so on until the full sequence was learned.

The rat had been taught in a similar way to press one lever five successive times, and then press another lever to obtain food. Both animals behaved as they had been taught despite the strange surroundings and the audience: and provided a most convincing demonstration that suitable manipulation of reward could indeed control behaviour so as to produce exceedingly complex learned actions.

Dr. L. Weiskrantz (University of Cambridge) emphasized the importance of such behavioural techniques, but he was concerned with the brain and with its relation to behaviour rather than with purely external observations of animals. Historically, the cortex of the brain had been regarded as composed of projection areas through which messages entered and left, and association areas which were more general in function. Indeed, according to Lashley's principle of mass action, the amount of deterioration, in performance in mazes, produced by removal of association cortex from a rat's brain depended on the weight of cortex removed and not the location. More recent research had shown both that the projection areas were less specific and that the association areas were more so than had been thought. Removal of the projection area which was fed by the ear had little effect on the ability of an animal to detect sounds. On the other hand, damage to the frontal association areas would produce an impairment in the ability to remember, over a short period, which of several possible hiding places contained food. The latter impairment did not occur with damage elsewhere in the brain. Similarly, damage to the temporal lobes would affect the ability to discriminate between two stimuli whereas damage to the frontal lobes would not. Thus different parts of the brain do have different functions.

Experiment in this area is made difficult by the irreversible nature of a lesion in the brain: once the frontal lobe has gone the animal cannot be tested again in its normal state. Recent work at Cambridge had, however, explored the possibility of temporary interference with particular regions by electrical stimulation, and it seemed that the ability to make delayed responses to hiding-places could indeed be removed temporarily while stimulation was being applied in the frontal lobes. The animals were afterwards normal in their performance. Once again different parts of the brain showed different effects.

Whereas the classical view of the nervous system was of a series of levels, in which the lower mechanisms dealt with isolated actions and higher ones with combined and co-ordinated ones, a different interpretation was now gaining support. On this view the nervous system might rather be compared with a candle in which the wick dealt with gross and undifferentiated action of the whole organism, and the outer skin of the candle dealt with finely discriminated functions. To take another analogy, on the old view, lower levels might be compared with isolated instruments and the cortex to the whole orchestra; on the new view, the contrast was rather between the orchestra tuning up, all simultaneously, and the orchestra playing in complex harmony.

Dr. Weiskrantz ended with a clear and well-reasoned argument for a physiological approach to behaviour as well as the approach in which actions alone are studied without reference to events within the animal; and as well as the approach in which mechanical models are constructed without physiological reference. He regarded these approaches as healthy in their scepticism, but negative, and firmly supported the importance of the brain in behaviour. It may be added that his paper was both witty and modest, and that it produced a deep impression on the audience.

Dr. R. Stretch (Queen's University, Belfast) gave a general review of experiments on the early experience of animals, including a few which show effects of ante-natal experience. It appears that exposure of pregnant rats to frightening situations may produce offspring which are unduly susceptible to fear: possibly through some ill-understood hormonal mechanism. Most research has, however, concentrated on early post-natal experience, and in this area the relationships seem to be complex. Electric shock administered to rats during infancy seems, for example, to produce adults which are more resistant to emotional disturbance. On the other hand, if animals are reared in a very restricted environment they seem as adults to be poor in ability to learn. There is some evidence that exposure to a complex environment in infancy produces adults with a lower concentration of cholinesterase in the brain, and this may be related to the differences in ability. There are also genetic differences as well as those produced by environment, and in this connexion it is interesting that wild rats differ from laboratory rats in the extent to which they will show curiosity or exploration. Wild rats avoid novel objects, while laboratory animals will investigate them and even feed in their presence. There may therefore be some interaction between anxiety and curiosity, and the effects of early experience on curiosity are of interest.

Dr. Stretch then turned to some experiments of his own on this latter problem. One group of rats was kept in ordinary laboratory wire-mesh cages for the period just after weaning, while another group of rats was kept in restricted cages through the walls of which they could not see. In the latter case there was little background noise, and the environment was in general a rather unstimulating one. As adults the rats were tested for curiosity in two ways. One of these was a maze shaped like an *H*, the measure of exploration being derived from the number of parts of the maze entered. On this test the animals which had had a restricted environment explored more. Another test could, however, be applied. For this a box was used, in an alcove of which novel cards with patterns on them were placed, and a record was kept of the extent to which the rats entered the alcove rather than the rest of the box. On this test the restricted rats gave a lower score. As Dr. Stretch pointed out, these results suggest that rats with a restricted early experience were more fearful. They therefore avoided particular novel objects, but if placed in a completely novel situation ran around looking for a means of escape. There are therefore

implications both for the methods to be used in studying curiosity and also for the possible mechanism by which variety of early experience has its effect.

The last speaker, Dr. J. M. Cullen (University of Oxford), was a zoologist rather than a psychologist. Accordingly he gave more attention to the natural behaviour of animals than did the other members of the symposium. His particular interest was the possibility of separating different classes of behaviour as affected by one drug rather than another. By this means one might ultimately disentangle the mechanism by which drugs affect behaviour. The animal which he had studied was the stickleback, and the drug benactyzine.

The stickleback lives in schools in the winter, but in the breeding season each male defends a territory in which it builds a nest. It courts an approaching female, she lays her eggs in the nest, departs, and the male then looks after the eggs. When defending the territory, the male will attack other males close to his own nest, but flee from them at greater distances. The border-line between these two behaviours moves nearer to the nest under benactyzine, which suggests at first sight that fear is increased.

When the female appears, drugged males less frequently court her, and are more likely to attack her than undrugged males are. This is not altogether consistent with the effect on size of territory, but might perhaps be explained by supposing that sexual behaviour is even more sharply reduced than fear is. On the other hand, kicking the tank in which the fish live causes them to cluster into a school, which suggests that schooling is a fear response: and the drug reduces schooling rather than increases it.

Feeding is reduced by frightening stimulation and also by the drug: but closer examination shows that the effects are rather different. In feeding, the stickleback orients towards the food, possibly several times, before each grasp at it. When frightened there are fewer grasps but also fewer orientations per grasp; whereas under benactyzine there are fewer grasps with a normal number of orientations per grasp. The latter type of behaviour also results simply from reduced hunger, so that the drug is in this respect apparently similar to a reduction in hunger.

The common factor in all these effects might perhaps be a reduced responsiveness to visual stimulation, and in fact sticklebacks placed in a situation where a striped wall rotates around them are less responsive to this stimulation. At the moment the most promising hypothesis is therefore that the drug reduces responsiveness rather than the strength of one category of behaviour; but the experiments remain an illustration of the way in which a knowledge of instinctive patterns may be used to analyse the operation of drugs.

It was clear from all four papers that work on animal behaviour is developing rapidly, and that important principles are beginning to emerge.

D. E. Broadbent

NEWS and VIEWS

Pharmacology at St. Bartholomew's Hospital Medical College: Prof. J. P. Quilliam

Dr. J. P. Quilliam, at present reader in pharmacology in the University of London at St. Bartholomew's Hospital Medical College, has been appointed to the newly created chair of pharmacology at that Medical College as from October 1. Dr. Quilliam graduated with honours (physiology) in the University of London, and took his M.Sc. in 1938, and his M.B., B.S. (London) in 1941. After a period of war service, in the Royal Air Force, he was appointed lecturer in pharmacology at King's College, London, and in 1946 he was appointed part-time lecturer in pharmacology at St. Bartholomew's Hospital Medical College to assist in the early development and expansion of that Department. In that early development he played a very active part and was appointed senior lecturer in charge of the Department and later reader in pharmacology in 1958. Dr. Quilliam has built up at St. Bartholomew's a very active Pharmacology Department with wide research interests and comprehensive teaching courses for medical, dental and science students. He has developed a department which is particularly well equipped for the use of electro-pharmacological techniques for research in pharmacology and for the teaching of that subject. He has built up a very strong and enthusiastic research team, with many postgraduate workers in his group, and he has attracted for his work important and valuable research grants from the United States. His research interests are manifold and his particular interests have centred on neuromuscular and ganglionic transmission and its modification by centrally-acting drugs. He has had wide experience of teaching methods, and he has spent a year as a Fellow in residence at Johns Hopkins University, Baltimore. He has had much experience as an examiner in the University of London and other universities in Britain, and is the chairman of the Board of Studies in Pharmacology in the University of London. He is a member of the General Optical Council. He has carried out important editorial duties as an editor of the *British Journal of Pharmacology and Therapeutics*, and his own publications involve investigations on the pharmacological action of hypnotic drugs, fluorophosphonates and many other drugs.

New Zealand Department of Scientific and Industrial Research: Senior Research Fellowships

The New Zealand Department of Scientific and Industrial Research has announced the award of the 1961 Senior Research Fellowships to Dr. B. H. Howard, principal scientific officer, Rowett Research Institute, Bucksburn, Aberdeen, Scotland, and to Dr. R. Melville, principal scientific officer, Royal Botanic Gardens, Kew, Richmond, Surrey, England. Dr. Howard's work has been concerned since 1954 with the study of the biochemistry of micro-organisms of the rumen, under Dr. D. P. Cuthbertson, director of the Rowett Research Institute. Prior to that he was studying biochemistry of micro-fungi, under Prof. H. Raistrick at the London School of Hygiene and Tropical Medicine. He has published several papers, and in his work under the Fellowship in New Zealand he hopes to apply techniques used in the United Kingdom to the different ruminal flora and fauna found in New Zealand ruminants. Dr. Melville is in charge of the Australasian Section of the Herbarium at Kew and is working mainly on Australian plants and to some extent on New Zealand plants, in

particular Ranunculaceae, Epacridaceae and Rutaceae. He has published several papers, in particular on *Ulmus*, and is very interested in the application of biometrical methods to taxonomy and the evolution of the angiosperm flower. He will be working in the Botany Division of the New Zealand Department of Scientific and Industrial Research.

Zoological Society of London : Senior Research Fellowships

Dr. I. W. Rowlands has been appointed by the Zoological Society of London as Senior Research Fellow in reproductive physiology. He will take up his appointment on January 1, 1962, and will be in charge of the Wellcome Institute of Comparative Physiology which is to be built in the Society's grounds next year. For the past twelve years Dr. Rowlands has been on the staff of the Agricultural Research Council and has worked at the Institute of Animal Physiology, Babraham, Cambridge, on problems concerned with the ovarian cycle of mammals. Before this, he worked at the Wellcome Veterinary Research Station, Frant, Sussex. Dr. Rowlands began his research career at the University College of North Wales with Prof. F. W. Rogers Brambell and was afterwards with Dr. A. S. Parkes at the National Institute for Medical Research in London.

Research Fellowships and Studentships

Dr. G. H. Manley, who is at present on the staff of the Department of Zoology, University of Nottingham, has been appointed to a research fellowship, and will take up his appointment on January 1, 1962, working initially with Prof. A. St. G. Huggett at St. Mary's Hospital Medical School, on reproductive behaviour of the lower primates. Mrs. A. D. Woodhead, who was been appointed to a research fellowship, has already taken up her appointment, and will work at the Fisheries Laboratory, Ministry of Agriculture, Fisheries and Food, Lowestoft, on the physiology and reproduction of fish. Mr. D. B. A. Symons and Mr. G. Glough have been appointed to research studentships.

Science at the British Council : Mr. E. Bolton King

Mr. E. Bolton King will retire from the posts of assistant controller, Arts and Science Division (Science Group), and director, Science Department of the British Council, at the end of this year. He will immediately thereafter take up the post of British Council science officer in Chile. Mr. Bolton King was educated at Warwick School and Balliol College, Oxford, where he was a mathematical exhibitioner and obtained first-class honours in physics finals. He was elected to a research studentship at Christ Church, Oxford, and carried out research at the Clarendon Laboratory, largely on photo-electricity. Later he formed the Oxford Instrument Co. Early in the war he joined the Ministry of Supply Rocket Team under Sir Alwyn Crow and was engaged on rocket development, with special emphasis on proximity fuses, investigation of the performance of British and enemy rocket weapons in the Mediterranean theatre, and liaison with rocket development in the United States, where he was an assistant director of the British Commonwealth Scientific Office. At the end of the War he went to Germany in connexion with the British firings of the V-2 rocket and became superintendent of the Ministry of Supply Establishment, Cuxhaven.

Dr. P. A. I. Tahourdin, O.B.E.

Dr. P. A. I. Tahourdin will succeed Mr. Bolton King as an assistant controller, Arts and Science Division, with responsibility for various departments, including the Science Department. Dr. Tahourdin graduated in physics at Oxford in 1943 and afterwards obtained a Ph.D. after nuclear work under the late Sir Francis Simon. He joined the British Council in 1946 and has served as science officer in Italy, assistant representative in Yugoslavia, and representative in Israel. He will assume his new duties early in 1962.

Mr. F. M. Beatty, M.B.E.

Mr. F. M. Beatty will become director of the Science Department in April 1962. He was trained as a physicist at Cambridge, taking his degree in 1940, and served during the War in the Royal Navy. He joined the British Council in 1946 and has served in the Science Department at headquarters, as science officer in Poland and in Austria, assistant representative/science officer in Brazil and, since 1959, as deputy representative in Pakistan.

Delegation from Academia Sinica in Britain

On the invitation of the President and Council of the Royal Society, a delegation from the Academia Sinica in Peking is visiting Britain during October 16–November 2. The members of the delegation are: Dr. Chu Ko-chen, vice-president and department member of Academia Sinica (meteorologist); Dr. Pei Shih-chang, department member and director of the Institute of Biophysics, Academia Sinica; Dr. Wang Shou-wu, deputy director of the Institute of Semiconductors, Academia Sinica; Dr. Chang P'ei-lin, deputy director of the Institute of Metals, Academia Sinica. Mrs. Chu is accompanying the delegation. Short visits are being made to Cambridge, Edinburgh and Oxford; and Dr. Chang is also visiting Sheffield. Visits will be made to university scientific departments, the National Physical Laboratory, the National Chemical Laboratory and other scientific institutes. On October 19 a special presentation and lecture on "Astronomy in Classical China" was arranged at the London Planetarium.

The Wellcome Trust: Grants for Research on Tropical Anæmias

The Wellcome Trustees are to provide up to £100,000 to assist an integrated programme of research by several groups of workers into the severe anæmias prevalent in tropical countries, with special reference to the malabsorption syndrome and tropical sprue. The Trustees already support work in this field by Dr. Henry Foy and Dr. Athena Kondi at the Medical Research Laboratories, Nairobi, Kenya, and Dr. Selwyn Baker and others at the Wellcome Sprue Research Unit at the Christian Medical College, Vellore, South India. Under the new arrangements the work of both teams will be associated with that of a new Wellcome Laboratory of Tropical Hæmatology which is to be established in Prof. J. V. Dacie's department at the Postgraduate Medical School of London, Hammersmith. The total allocation of £100,000 for the work on tropical anæmias and kindred disorders is made up as follows: £45,000 to build and equip the Wellcome Laboratory of Tropical Hæmatology at Hammersmith; £14,000 to organize and maintain a scheme of close co-operation between

the groups led by Dr. Foy, Dr. Baker, Dr. D. L. Mollin of the Hammersmith Laboratory, and a Royal Army Medical Corps team in Singapore; £25,000 for the continued support of Dr. Baker's unit in Vellore; £15,000 for new X-ray equipment at Vellore.

Awards in the United Kingdom and the United States

Other major grants in aid of research out of the total of £248,00 distributed by the Trust during the past half-year included: £40,000 to Prof. H. O. Schild and others for the extension to the Pharmacology Building at University College, London; £15,000 to Prof. J. D. Lever to purchase an electron microscope for the Department of Anatomy, University College of South Wales and Monmouthshire, Cardiff; £13,500 to Prof. D. H. R. Barton for the purchase of a nuclear magnetic resonance spectrometer for the Department of Organic Chemistry, Imperial College of Science and Technology; £10,500 to Dr. D. P. Cuthbertson for the purchase of new radiographic equipment for the Rowett Research Institute, Aberdeen; £10,000 to Prof. H. E. de Wardener, Department of Medicine at the Fulham branch of Charing Cross Hospital; £9,850 to Prof. F. W. Rogers Brambell to increase the accommodation for post-graduate research workers in the Department of Zoology, University College of North Wales, Bangor; £4,000 to Dr. A. H. Harthoorn, to provide a mobile laboratory for field research in comparative physiology, University College of East Africa, Nairobi. A five-year senior Wellcome research fellowship has been provided for Dr. Karlis Adamsons in the Columbia-Presbyterian Medical Center, New York City, at a cost of 50,000 dollars. His work will be concerned chiefly with the physiology of the new-born.

New Member of Trust

The Wellcome Trust announces that Mr. Lancelot Claude Bullock, who was appointed a trustee under the will of the late Sir Henry S. Wellcome, has retired. For many years he was senior partner in the London firm of solicitors, Markby, Stewart and Wadesons, where his associations with Sir Henry Wellcome go back to the time he was asked to advise and draft the will under which the Trust was created. He is succeeded by Mr. Robert Malleson Nesbitt, also of Markby, Stewart and Wadesons.

The State and the Freedom of the University

Prof. M. Bouchard, writing in *Science and Freedom* (No. 19; June 1961) on "The Universities in France: Freedom and Autonomy", expresses some concern at the effect on university autonomy of the growing dependence of the universities on State support. While the maintenance of university freedom depends in the last resort on the moderation and liberal outlook of the State, Prof. Bouchard is no less concerned that the individual members of the universities must be allowed full scope for their inquiries and research in whatever field they please, without let or hindrance. They must be free to form their own conclusions and say what they believe to be true. In France, university teachers have the status of Civil servants and are protected from all arbitrary measures by a special legal clause which offers security so long as the spirit of independence is kept alive in the universities, and Prof. Bouchard pleads that care should be given to the preservation of this vital principle. To the same issue, Prof. L. Massart contributes a note on the problems and prospects of the Belgian universities. Pointing out that the appointments of teaching staff are not immune from political influence, he stresses, however, that the real problems of the universities in Belgium arise out of their growing dependence on State support and the increasing number of students. He is disturbed at the persistence of obsolete methods of teaching, the failure to increase the number of students trained for research commensurately with the national needs and the effects of inbreeding from which the university system suffers. He believes that a thorough reappraisal of the university structure in Belgium is required. While the number of students has increased from 10,307 in 1938–39 to 26,057 in 1958–59, and the number of graduates from 1,995 to 4,930 over the same period, the number of doctorates has only risen from 79 to 120, a 40 per cent drop in the rate per 100 graduates.

The Berkshire, Buckinghamshire and Oxfordshire Naturalists' Trust, Ltd.

The first annual report of the Berkshire, Buckinghamshire and Oxfordshire Naturalists' Trust, Ltd., which was incorporated as a limitod company on January 9, 1961, covers the year 1960 (Pp. 7. Kennington, Oxford: Berkshire, Buckinghamshire and Oxfordshire Trust, Ltd., 146a Poplar Grove, 1961). At its incorporation the Trust had 328 members, and the major task of the Provisional Executive Committee during the first year was the preparation of a draft constitution which was approved at a meeting of members on November 12, 1960. The objects of the Trust, as set out in its memorandum of association, are to record and investigate places and objects of botanical, zoological, geological, archæological or scientific interest, or of natural beauty, and to protect these from injury, ill-treatment or destruction; to establish, form, own, maintain and manage nature reserves or bird sanctuaries; to encourage the breeding of wild-birds, insects and plants which are harmless, useful, beautiful or rare; and to promote, organize, carry on and encourage study and research for advancing knowledge in the natural sciences. The Trust has already been concerned with threats to several important sites. In December 1959 it joined with the Nature Conservancy and other bodies in successfully resisting the proposal to build a 250-ft. radio tower in Little Lenham Wood, near Beacon Hill. It has also joined with the Banbury Ornithological Society in asking for the preservation of certain woods in that neighbourhood if further large-scale ironstone working is authorized, and has also sought to preserve the heronry at Chievsley Furze, the famous crocus field at Inkpen, Berkshire, and the sites on which the military and monkey orchids, the red helleborine and the wild fritillarias grow.

Museums Journal

The second issue of the new format of the quarterly *Museums Journal* (61, No. 2, September 1961) maintains the high standard and deals with a variety of topics. Mr. F. I. G. Rawlins emphasizes the value of the scientific outlook in conservation, Mr. Philip Rawson describes the new Gulbenkian Museum in the University of Durham and Mr. Robert Hill deals the various types of electronic lecture guide systems which are now proving of such absorbing interest to museum curators. Much of this issue is devoted to a

record of the annual conference of the Museums Association held at Plymouth in June 1961. A report on the symposium held at the British Museum (Natural History) on "The Museum and the Naturalist", and book reviews, complete an informative issue.

The Bernice P. Bishop Museum, Honolulu

UNDER the title of "Work Half Done" the Bernice P. Bishop Museum at Honolulu issues its report for 1960. The title refers to the fact that at the end of 1960 the Museum was in the middle of a particularly important project, the new Planetarium-Observatory. This ambitious building is being constructed without the necessary total of capital funds but with the conviction that these will be forthcoming when the value of the venture is demonstrated. An interesting experiment was involved in the trapping of air-borne insects to determine the role of air currents in insect dispersal. This was carried out both on board ship and by a special device in aeroplanes.

The Stanley Clifford Milk Industry Award

THE Central Milk Distributive Committee, in recognition of the valuable services rendered in the dairy industry by Stanley Clifford, who was for many years chairman of the Committee, has established a fund for the purpose of providing awards to enable a suitable applicant to make a short study tour of the dairying industry in the United Kingdom or abroad. Employees in the dairy industry or in dairy engineering in the United Kingdom who are below the age of thirty are eligible. The Award, which will be made annually, will be administered by the Society of Dairy Technology. It has been decided that the first award shall be granted for a study tour in Denmark immediately following the International Dairy Congress which is to be held in Copenhagen during September 3–7, 1962. Applications for this Award should be made to the Secretary, Society of Dairy Technology, 17 Devonshire Street, London, W.1, not later than January 31, 1962.

Royal Institute of Chemistry Lecture Series

THE first three numbers of the Royal Institute of Chemistry 1961 series of lectures have just been published (The Royal Institute of Chemistry. Lecture Series 1961. London: Royal Institute of Chemistry, 1961. 5*s*. each). The first, entitled *Atmospheric Photochemistry*, is by Sir Harrie Massey and Dr. A. E. Potter; the second, *A Chemist's Introduction to Statistics, Theory of Error and Design of Experiment*, by Dr. D. A. Pantony; and the third, *Recent Developments in Polarography*, is by Dr. G. W. C. Milner. The booklets also contain a list of other recent publications of the Institute.

The Night Sky in November

NEW moon occurs on Nov. 8d. 09h. 59m. U.T. and full moon on Nov. 22d. 09h. 44m. The following conjunctions with the Moon take place: Nov. 2d. 05h., Regulus 0·01° N.; Nov. 13d. 13h., Saturn 3° S.; Nov. 14d. 00h., Jupiter 3° S.; Nov. 23d. 03h., Aldebaran 0·7° S.; Nov. 29d. 13h., Regulus 0·3° S. In addition to these conjunctions with the Moon, Venus is in conjunction with Spica on Nov. 4d. 17h., Venus being 4° N. Mercury is a morning star, visible low in the south-east before sunrise; on November 7 it rises almost two hours before the Sun. Venus is a morning star, rising at 4h. 50m., 5h. 30m. and 6h. 20m. on November 1, 15 and 30, respectively. It is rather too close to the Sun for convenient observation. Mars is too close to the Sun for observation. Jupiter and Saturn are evening stars, setting about four hours after the Sun. They may be seen low in the south-west after sunset; they are not very favourably situated for observation. Occultations of stars brighter than magnitude 6 are as follows, observations being made at Greenwich: Nov. 20d. 16h. 56·9m., ξ^2 Cet. (*D*); Nov. 21d. 2h. 22·9m., μ Cet. (*D*.); Nov. 23d. 2h. 52·8m., α Tau. (*D*); Nov. 23d. 3h. 44·3m., α Tau. (*R*); Nov. 24d. 4h. 23·5m., 119 Tau. (*R*). Nov. 24d. 5h. 07·4m., 120 Tau. (*R*); Nov. 24d. 21h. 14·4m., 71 Ori. (*R*); Nov. 27d. 3h. 56·7m., ζ Cnc. (*R*); *D* and *R* refer to disappearance and reappearance, respectively. The Taurid meteors are active during the first half of the month, maximum activity being on November 7. The radiant is at R.A. 3h. 36m., Dec. + 14°, and conditions for observation are favourable. The Leonid meteors are active on November 16, the radiant being at R.A. 10h. 08m., Dec. + 22°, and conditions for observation are favourable.

Announcements

PROF. W. T. WILLIAMS, professor of botany at the University of Southampton, has been appointed a member of the Agricultural Research Council in succession to Prof. S. J. Watson, who has retired after ten years service.

THE one-hundred-and-twenty-eighth meeting of the American Association for the Advancement of Science will be held in Denver, Colorado, during December 26–30. Further information can be obtained from the director of public information, Sidney S. Negus, Medical College of Virginia, Richmond, Virginia.

AN international conference on "Magnetic and Electric Resonance and Relaxation" is to be held at Eindhoven, The Netherlands, in July 1962, under the auspices of the Netherlands Physical Society and the *Groupement Ampère*. Further information can be obtained from Dr. D. J. Kroon, Philips Research Laboratories, Eindhoven, Netherlands.

THE second International Symposium on Continuous Culture of Micro-organisms will be held in Prague during June 18–23, 1962. It is being sponsored by the Czechoslovak Academy of Science. Further information can be obtained from Academician Ivan Málek, Czechoslovak Academy of Science, Institute of Biology, Na cvičišti 2, Prague 6, Czechoslovakia.

MESSRS. MACMILLAN regret that an incorrect price for Volume II of Partington's "History of Chemistry" was given in *Nature* of October 14, p. lxxxix; the volume is being published at £5 5*s*. net.

ERRATA. Dr. D. H. Trollope has written stating that some omissions and misprints have occurred in the communication entitled "Effective Contact Stresses and Friction" published in *Nature* of July 22, p. 376. Equation 7 should read: $R = \frac{W}{N} - ua + \rho a$; equation 8 should read: $Fj = \mu\left(\frac{W}{N} - ua + \rho a\right) = \mu\left(\frac{W}{N} - u\frac{A}{N} + \rho\frac{A}{N}\right)$; equation 9 should read: $S = \mu(\sigma - u + \rho)$. On p. 376, col. 2, line 41. for 'macro-fraction' read 'micro friction'; line 56, for 'invert' read 'inert'; line 57, for $\mu = 0$ read $u = 0$.

SCIENTISTS IN THE PUBLIC SERVICE

SPECIAL PROMOTIONS

As in previous years a number of special posts has been created in the Scientific Civil Service under the provisions included in the White Paper on the Scientific Civil Service (Cmd. 6679, 1945) to provide for the promotion of individual research workers of exceptional merit. The promotions were effective from July 1 and include the following :

Deputy Chief Scientific Officers

Dr. L. J. Bellamy, who is superintendent of Analytical Services at the Ministry of Aviation's Explosives Research and Development Establishment, Waltham Abbey, entered Government service in 1939 and undertook various analytical jobs until he was posted to the Directorate of Chemical Inspection, Woolwich, in 1945. Here he became responsible for the development of new methods of analysis, particularly physical methods, and became a member of the World Health Organization Expert Advisory Committee on Insecticides. In 1955 he was transferred to his present post, where he has been responsible for the development of new analytical methods, research and assessment on the stability and compatibility of explosives, X-ray and electron microscopy techniques and polymer synthesis and properties. Dr. Bellamy's major personal contributions have been in the fields of the interpretation of the spectra of large molecules, solvent effects, and more recently on the difference in the associative properties of protons and deuterons. On the interpretation of spectra of large molecules, Dr. Bellamy has developed a broad theory, whereby the behaviour of the more important group frequencies can be predicted and understood. This has revealed new relationships between frequency shifts and other physical properties. On solvent effects, his work has led to a better understanding of inter-molecular forces in solution.

Dr. A. F. Gibson is leader of the Semiconductor Physics Research Division at the Royal Radar Establishment of the Ministry of Aviation at Malvern, and has been associated with that establishment since 1944. Dr. Gibson's work has included luminescence, infra-red photo conductors, transistor physics and research of a fundamental character on the properties of semiconductors. He has applied his research to practical needs and has been responsible for the development of a new form of far infra-red and millimetre wave detector, based on cyclotron resonance and an avalanche injection diode of value in fast-switching circuits. He has carried out investigations of the microwave properties of germanium and the effect of carrier injection and extraction on the absorption. Dr. Gibson's most recent work has been concerned with 'hot' electrons in germanium, which has shown the complex nature of the processes whereby energy given to electrons in a semiconductor by an electric field is exchanged with the lattice to give ohmic heating and how, when this cannot be done sufficiently rapidly, the energy is spread unequally among the various 'valleys' in momentum space. Similar investigations carried out with 'holes' have led to a better understanding of the band structure of the complex valency band of germanium.

Senior Principal Scientific Officers

Dr. J. M. Burch, of the National Physical Laboratory, specializes in metrological optics, particularly in the production and application of photographic gratings. These gratings are of two kinds: large photographs of very closely spaced interference fringes for spectroscopic purposes, and gratings for linear and radial measurement based on the improvement of conventional rulings by methods of 'photographic averaging'. Gratings, read by optical and electronic methods to which Burch has also made important contributions, are now being extensively used in the control of machine tools and in metrology. Dr. Burch has recently taken charge of a research project concerned with the development and application of optical masers.

Prof. J. C. Cooke, one-time professor of applied mathematics in the University of Malaya, is now working on fluid dynamic theory in the Aerodynamics Department at the Royal Aircraft Establishment, Farnborough.

Dr. D. A. L. Davies, attached to the Immuno-Chemistry Section of the War Department's Microbiological Research Establishment at Porton, is concerned with work on the isolation and structure of bacterial antigens. His work has laid the basis for a chemical and immunological approach to tissue-cell antigens.

Mr. D. Firth is now in charge of the work of the Fluid Power Section of the National Engineering Laboratory concerned with research into oil hydraulic and pneumatic devices for general industrial use. Earlier this year Mr. Firth won the Wolfe Award for his work on hydrostatic transmissions.

Mr. J. F. Gittins, of the Services Electronics Research Laboratory Extension, run by the Admiralty at Harlow, has undertaken research in the field of high-power microwave valves, particularly of the travelling-wave variety. This has led to the use of the travelling-wave tube as an effective high-power amplifier at microwave frequencies. Mr. Gittins is also chairman of the Power Valve Research Advisory Panel of the Co-ordination of Valve Development Committee.

Mr. B. E. J. Pagel is in charge of the Astrophysics Department at the Royal Greenwich Observatory. In addition to carrying out theoretical and observational research Mr. Pagel has also assisted in the design of new apparatus and introduced improved techniques.

Dr. G. B. Preston, of the Royal Military College of Science, Shrivenham, has been engaged in research in the field of pure mathematics, particularly in the theory of semi-groups.

Dr. J. T. Stuart, of the Aerodynamics Division, National Physical Laboratory, has made contributions to the study of the stability of fluid flow. Of particular significance is his research work into the non-linear theory of hydrodynamic instability, the growth of wave disturbances and the effects of Reynolds stresses on transition from laminar to turbulent flow.

Dr. R. H. Wood is in charge of that part of the Building Research Station research programme that

deals with structural engineering design problems; his particular interest is the structural interaction between the various elements of construction in multi-storey framed buildings. This interaction, or mutual stiffening of the parts of a building so as to strengthen the whole, has an important influence on building design, and Dr. Wood's work has contributed greatly to the understanding of the structural behaviour of complete buildings and to the more efficient allowance for composite action in structural design.

Similar promotions have been made by:

(1) United Kingdom Atomic Energy Authority: Senior Principal Scientific Officers

MR. J. C. MARTIN has worked at the Atomic Weapons Research Establishment, Aldermaston, since its foundation in 1953. His early work was concerned first with small-scale explosives research, investigating the motion of fluid surfaces and later with the development and use of scintillation counters. More recently he has worked on neutron sources and on the development and application of sub-microsecond radiographic techniques for the experimental work on explosions. Starting with an early prototype 35-MeV. linear accelerator for which he developed a double focusing system, he has brought it to a state of output and reliability which has made it a valuable tool in the solution of certain hydrodynamic problems. He is now attempting to extend the flash X-ray technique from its present limit in the region of 1 MV. up to about 6 MV. He has contributed a number of original ideas in the field of advanced nuclear and thermo-nuclear weapons.

DR. J. B. TAYLOR has worked at the Atomic Weapons Research Establishment, Aldermaston, for six years. He contributed first to the improvement of neutronic calculations used in the theory of explosions and in the computation of the stochastic behaviour of small populations in a supercritical system. He then carried out a number of investigations, ranging over several fields and at all levels of detail, to illuminate and help to direct the astrophysical engineering behind the megaton test series of 1957–58. He later worked on problems connected with fast reactors and on electromagnetic phenomena from explosions, before turning to problems of the thetatron and other controlled thermonuclear reactors.

(2) Agricultural Research Council: Deputy Chief Scientific Officer

DR. R. MARKHAM joined the staff of the Plant Virus Research Station, Cambridge, now the Agricultural Research Council Virus Research Unit, in 1940 and became director of the Unit in 1960. His research has been concerned mainly with the biochemistry of the smaller plant viruses, and in 1949 he was able to demonstrate that the ribonucleic acid of the turnip yellow mosaic virus, which he and Dr. Kenneth Smith first isolated and purified, was necessary for the infectivity of the virus and was enclosed in a protective shell of protein. This work led to an intensive investigation of nucleic acids in general and resulted in the development of a large number of delicate methods for the analysis of nucleic acids and their constituents. It also led to an understanding of the way in which some of the enzymes which degrade nucleic acids, particularly pancreatic ribonuclease, act. Dr. Markham has also been concerned with synthetic mechanisms by which small polynucleotides can be made. He was elected a Fellow of the Royal Society in 1956.

Senior Principal Scientific Officers

DR. C. H. CADMAN is head of the Virology Department, Scottish Horticultural Research Institute, Mylnefield, Dundee, and is at present particularly concerned with the transmission of plant viruses by nematodes.

Dr. B. KASSANIS, of the Plant Pathology Department, Rothamsted Experimental Station, has discovered and described a number of viruses, and recently discovered that the multiplication of the virus depends on the simultaneous multiplication of another.

Dr. A. KLECZKOWSKI, of the Plant Pathology Department, Rothamsted Experimental Station, is primarily a serologist, but has made substantial contributions to a range of subjects, and is now studying the surface structure of the protein units which make up tobacco mosaic virus.

THE ROYAL SCOTTISH MUSEUM

By DR. D. A. ALLAN, C.B.E.
Formerly Director

THE foundation stone of the Royal Scottish Museum was laid by the Prince Consort in October 1861, and thereafter there arose in instalments the Venetian Renaissance building which now occupies more than half of Chambers Street, west of the Old College, University of Edinburgh. It is a grey stone structure consisting of a central block elongated east and west, with two wings stretching southwards. The area within was afterwards built on to yield a series of rectangular halls, most of them with a main ground-floor surmounted by two balconies, the earlier ones supported on cast-iron pillars, the later either cantilevered or upheld by ferro-concrete structures. A 'grand staircase' was added at the rear in 1931, with arches at its mezzanine landings to lead to a prospective future block. A lecture hall had been included in the earliest part of the building but was later sacrificed to yield much-needed exhibition space, yet the necessity for a hall for lectures and demonstrations and later to provide educative film shows was ever present, and just before the Second World War foundations were dug beyond the 'grand staircase' for a rectangular lecture hall . . . and were left to be obliterated by ashes from the boilers. In May 1959 work was commenced on a theatre block of an entirely novel design, and its completion was achieved early in September 1961, the opening ceremony being performed by the Right Hon. the Earl of Rosse, chairman of the Standing Commission on Museums and Galleries, with the Right Hon. John Scott Maclay, H.M. Secretary of State for Scotland, in the chair.

XUM

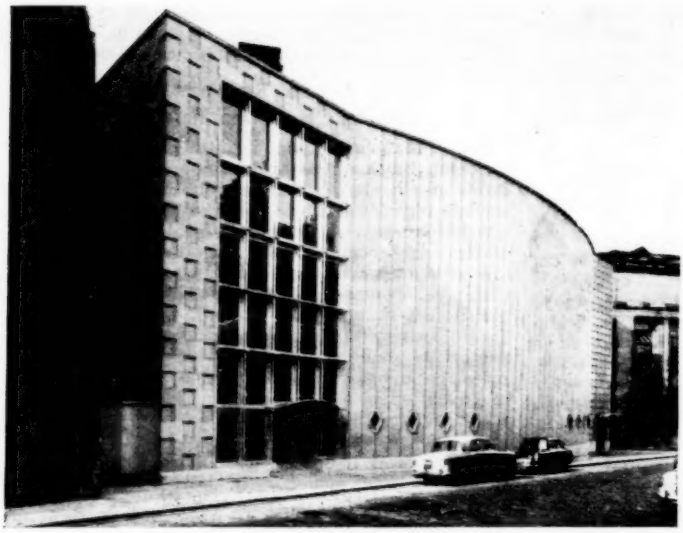

Fig. 1. The Royal Scottish Museum, New Theatre Block

The site was a difficult one, being a triangle hemmed in by part of the back of the old building, a busy street and a little cul-de-sac. Again, whatever occupied it had to marry with a 1914 red sandstone somewhat classical façade of the Museum zoological galleries beyond and the grey sandstone of the Robert Adam Old College building further on.

The principal contribution being a lecture theatre, it had to be given the lion's share of the space, and the angle of the seating floor was determined by the number of persons to be accommodated. As the Museum's opening ceremonies attract around 200 guests—and the early 1900 public lectures, organized by the Museum but housed in the adjacent University examination hall, as many as 250—it was determined that the new theatre should hold 300. The plans allowed for this number of seats in ten curving rows of thirty, rising steeply on steps, with three aisles. The platform is low, long and narrow, so that everyone can have an uninterrupted view of speaker, demonstrations or of the screen occupying the central portion of the wall beyond. The back of the auditorium is against a solid bow-shaped, windowless street wall, impervious to light and sound. At the narrowing end of the triangle is an emergency staircase, with a side door at street-level. The wider end of the triangle is a three-storeyed block, each floor consisting of a curving chamber running from the street back to the older part of the building. The ground-floor is a foyer with a door to the street, another to the main museum building and the double sound-proof doors to the front of the auditorium. Above are two more floors, reached by an open staircase of steps cantilevered on a single supporting beam, with landings against an immense window rising the height of the building and facing the street. Because of differences in level, the foyer is broken across the middle and a short flight of curving steps leads from the new end to the old. The top-floor gallery opens on to an extensive exhibition hall over the lecture theatre, top lit and with an additional exit to the top of the emergency staircase. The proportions of the building can be put briefly and roughly—two-thirds width and two-thirds height for the lecture theatre, with the remaining third at the top exhibition hall and the remaining third at the side foyer with two overlying exhibition rooms. Underneath is a large cellar space, which will later be developed to advantage for much-needed storage. In contrast to the prevalence of right angles throughout the many halls of the old building, those in the new are limited to the contacts between floors and walls and walls and ceilings.

The lecture hall, which will be used for opening ceremonies connected with special exhibitions in the adjacent halls, conferences, lectures, school-class talks and cinema shows, presents a novel, modern and entirely charming interior, with its major sweep across its width, and the steeply tiered rows of tip-up chairs, upholstered in grey plasticized fabric, flanked and backed by walls lined to the full height with *Abura* wooden strips of a warm brown colour. The ceiling, which is also acoustically designed, presents a pattern of shallow rectangles in relief to the audience but projects a battery of lights from the unseen forward and downward projecting ends on to the platform. The lighting can be controlled either from the stage or from the projection room in the rear of

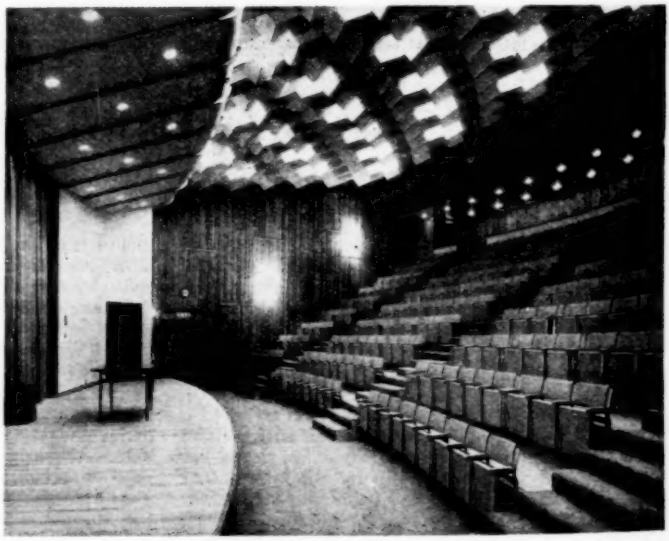

Fig. 2. New Lecture Theatre, Royal Scottish Museum

the ceiling. The tiers and access gallery at the back are covered in grey marbled plastic flooring, with gold-coloured carpet on the three access stairways and blue carpet fronting the stage, the curtains of which are in gold. Hardwood strip has been used in the floors of the exhibition halls and travertine for the foyer. The 'flying' staircase is finished with hardwood treads with rubber inserts and aluminium; plate glass and plastic have been used for the balustrade. The architect has designed an appropriately carved teak door for the emergency exit. The designs begin at the base with early forms of life and fossils, pass up to various animals and man, show locomotives, telephones and television sets, and lead to a top row of aeroplanes, sputniks and an atomic explosion. The 'finale' is a row of question marks.

The exhibition halls are heated by low-pressure water coils contained within the floor. Background heating for the theatre is provided by coils behind the stage front and this will be supplemented when the hall is in use by warmed air introduced through the slats on the walls near the stage and extracted at the ceiling below the projection room. The 16-mm. projector is sited in a special room reached by a passage above and behind the auditorium. The building could well have taken a full-size projector, but in view of the difficulty of getting documentary films suitable for educational purposes, the smaller size had to be chosen.

The façade of the new extension is spectacular and modern, yet it provides a pleasing gradation with its neighbours. The bastion-like central mass consists of plain ribs of cream-coloured sandstone, reminiscent of a clinker-built boat, and standing on a simple polished granite base. In the lower portion of the sandstone are a few ovoid windows.

The curved central mass is flanked by two unequal flat walls, that to the east being smaller and having its square sandstone blocks split into two facets angled diagonally, so as to yield an attractive shadow pattern. The greater flank to the west is framed in square sandstone blocks with alternate ones set back to provide a different pattern, but the main face is a rectangular aluminium frame with a slightly arched doorway in the centre surmounted by five tiers of plain glass panes, the horizontal crossings marked by narrow bars which combine decoration with utility in supporting window cleaners. Through the glass, the stair landings add a decorative motif. The architect was Stewart Sim, a senior architect of H.M. Ministry of Works, and the contract was carried out by Thaw and Campbell, Ltd., Glasgow, at a cost of approximately £120,000. Thus the Royal Scottish Museum displays the march of time in its architecture and its furnishings—in its exhibits as well—over a full century.

CANCER RESEARCH IN THE UNIVERSITY OF PERUGIA MEDICAL SCHOOL

By Prof. LUCIO SEVERI
Director

ON June 26, the occasion of the centenary celebrations of the chair of morbid anatomy of the University of Perugia, Dr. Thelma B. Dunn, head of the Cancer Induction and Pathogenesis Section, National Cancer Institute (U.S.A.), opened at Montelucе an extension to the Division of Cancer Research, University of Perugia Medical School.

The ceremony included a visit to the new animal-house and the remodelled older one, on the main floor, in which normal work was in progress. The guests consisted essentially of the participants of the international conference on the "Morphological Precursors of Cancer". At the opening of the extension, all the doctors *honoris causa* of the University of Perugia Medical School were present, including Prof. A. Haddow, director of the Chester Beatty Research Institute, London; Prof. J. W. Orr, director of the Department of Pathology and Cancer Research Laboratories, University of Birmingham; Prof. J. J. Bittner, Division of Cancer Biology, University of Minnesota, Minneapolis; Dr. L. C. Strong, director of the Biological Section, Roswell Park Memorial Institute, Springville; Dr. J. R. Heller, president of the Memorial Sloan–Kettering Cancer Center, New York; Prof. C. Krauspe, director of the Institute of Morbid Anatomy, University of Hamburg; Prof. V. R. Khanolkar, vice-chancellor of the University of Bombay; and Prof. T. Yoshida, dean of the Faculty of Medicine, University of Tokyo.

The extension was designed by Prof. G. Nicolosi, Rome, and the work was carried out under the direction of Dr. G. Castori, Perugia, being erected by Messrs. Fioroni, Perugia, at a cost of 50,000,000 lire. The new accommodation doubles the previous floor area and makes the Division the largest and best equipped organization of its kind in Italy.

The extension consists of four storeys of concrete-cased steel-framed structure, faced with grey-coloured plaster. The various floors are formed with pre-cast concrete flooring units. The various services to the laboratories, which include hot and cold water, gas, electricity, compressed air, vacuum and steam, are visible, not concealed. In this way easy access is possible to the various services and pipe-lines when it is desired. Each floor is served by an automatic electrically operated passenger lift. The new building is linked with the older Division of Cancer Research by an underground passage, and contains extensive animal-house accommodation and laboratories for virology, tissue culture and cytogenetics.

The main financial responsibility of the Division of Cancer Research has always been borne by the University of Perugia. Additional funds are provided by the National Cancer Institute of the U.S. National Institutes of Health, and to a lesser, but valued. extent from the Lega Italiana per la Lotta contro i Tumori, Consiglio Nazionale delle Ricerche, Farmitalia Milano, and from other private Italian benefactions.

It is only since the Second World War that the idea of a Division of Cancer Research in the University of Perugia was conceived. But it was several years before the plan could be carried out. On November 30, 1952, the Minister of Public Education, on the occasion of the opening of the academic year, announced the establishment of a University Division of Cancer Research at Perugia. In the first building were established an animal

Fig. 1. New Extension of the Division of Cancer Research, University of Perugia Medical School

colony and laboratories for research in biology and pathology. Later, in the years 1956 and 1957, a programme of improvement was completed with the aid of grants and contributions.

During recent years, investigations have been carried out in the Division by various groups of scientists on different aspects of cancer research. Work on the spreading of metastases in human and experimental tumours has been described in a series of papers dealing with the various facets of the problem. A valuable contribution to the conflicting problems of the morphological precursors of human breast cancer was made by the staff of the Division of Cancer Research working as a unit. In the initial phase, different types of mammary cancer, arising from different lesions, and characterized by a different age at onset, ætiological pattern and morphology were detected and classified. It was then shown that the frequency and the latency period were also different for each of these types of cancer. On this basis, an attempt was made to determine the order of importance of the respective morphological precursors; this is still regarded as a useful factor for prognosis.

In recent years, much effort has been transferred to the study of causation, behaviour and evolution of mammary tumours in inbred mice. The origins, nature, fate and hormonal control of the hyperplastic alveolar nodules in the mammary glands of virgin and breeding animals carrying the milk factor have been analysed in a series of papers. After the discovery of a new kind of pre-malignant change, the plaque, in hybrid animals, various inbred strains of mice normally bred were investigated to determine the occurrence of plaques. These have been found in two of the three strains investigated. In connexion with these experiments, an analysis of responsiveness and progression of mammary tumours of inbred mice was carried out, which led to the detection of the existence of strain differences in the biological and morphological characters of neoplasms. Finally, a recent extension of these investigations has shown that the strain differences found might be due to differences in the properties of the respective milk factors.

A new field of investigation, cytology, opened at the Division of Cancer Research with the conversion of a spontaneous mammary tumour into an ascites tumour. Work on the mitotic rate and chromosomal pattern of this ascites tumour is now in progress.

Recently, it was demonstrated that isoniazid, a drug very widely used in the management of tuberculosis, is carcinogenic for the lungs of *BALB/c* mice. Due to its practical importance, this field of investigation has been rapidly extended in order to see whether isoniazid is carcinogenic to other species of animals, whether it is carcinogenic in itself or through some of its metabolites, and how this carcinogenic action may be prevented.

Since its foundation, the Division of Cancer Research has encouraged, within the limits of its facilities, visits of qualified scientists from foreign countries, in order to exchange information and ideas on the work in progress. A large number of such visitors have come during recent years. Several of them delivered lectures. For others, small seminars were organized with the staff. Other visiting scientists have spent several months working at the Division of Cancer Research with members of the staff, in fields of common interest.

THE INTERNATIONAL FEDERATION FOR DOCUMENTATION

THE twenty-seventh conference of the International Federation for Documentation was held at Caxton Hall, London, during September 6–16. The Federation has its headquarters at The Hague, and its work on many different problems of documentation is carried on mainly through correspondence, committees and meetings of the Executive Bureau and a General Assembly held each year in a different country. The large, unrestricted, conference gatherings take place only every three or four years and this was the first to be held in England since 1938. It was attended by more than 500 delegates coming from 38 countries and from all five continents. Aslib, as the British representative

body in the Federation, was responsible for the arrangements.

The earlier part of the conference, that is, during September 6–11, was devoted to meetings of committees, the Executive Bureau, and the General Assembly with a special session on September 9 to consider present-day systems and trends in automatic documentation such as the use of electronic computers and research into the possibilities of mechanical translation. Excursions to Oxford and Cambridge were arranged for September 10.

During the second week of the conference, an exhibition of new processes and experimental work in documentation was open, and included a device for the automatic identification of printed numerals demonstrated by Dr. M. B. Clowes and Mr. J. R. Parks of the National Physical Laboratory.

The official opening of the full conference took place on September 12 when the Baroness Wootton of Abinger, deputizing for Lord Hailsham, Minister for Science, gave the opening address. The chairman, Dr. Burton W. Adkinson, head of the Office of Science Information, U.S. National Science Foundation, referred to the great loss to the Federation through the recent death of Fritz Donker Duyvis. His passing had been "the quiet closing of a door on an age", the age of the pioneers of documentation such as Otlet and La Fontaine, and of the devoted amateurs who followed them such as "D. D." who, for more than two decades, was the embodiment of the International Federation for Documentation. After this tribute to the late secretary-general of the Federation, the meeting heard four papers describing recent developments in documentation. These were presented by Dr. K. Schneider (Germany), who described the use of electric typewriters operated by punched cards; Mr. A. C. van Schaik (Netherlands), who showed that advantages could come through bibliographical co-operation within a country; Mr. C. W. Cleverdon (United Kingdom), who outlined the work of the Aslib Cranfield investigation into the efficiency of indexing systems, and Mr. Karl F. Heumann (United States), who presented Verner W. Clapp's summary of recent important progress in documentation in the United States. In the evening, delegates attended a reception given by the Vice-Chancellor of the University of London and Mrs. Noble at the Senate House.

The morning sessions of September 13 dealt with scientific and technical information under the chairmanship of Dr. M. A. T. Rogers, research controller, Imperial Chemical Industries, Ltd., deputizing for Dr. T. E. Allibone, who was indisposed. Papers were contributed by Mr. R. G. l'Hermite (France), Dr. A. Moore (United States), who dealt particularly with the problem of information in the pharmaceutical industry, and Dr. Y. Niwa, who described the history and growth of the Japan Information Centre of Science and Technology and similar services in his country.

The afternoon session was presided over by Mr. G. Deurinck, of the Fondation Industrie-Université pour le Perfectionnement des Dirigeants d'Enterprises, Belgium. The papers covered the documentation of management (Prof. R. W. Revans, United Kingdom, and Prof. J. L. Mey, Netherlands), and the use of information for economic planning (Mr. K. Havliček, Czechoslovakia). In the evening delegates were invited to a reception given by Imperial Chemical Industries, Ltd., at Imperial Chemical House, Millbank.

The efficient use of information was the theme of the morning session for September 14 when the chair was again taken by Dr. Burton W. Adkinson. Mr. R. E. McBurney described the work of the Canadian Technical Information Service of the National Research Council. Mr. K. Klintoe, director of the Danish Technical Information Service, spoke on productivity in information service, Mr. William T. Knox gave an interesting talk on the use by some United States companies of senior scientists or engineers as information research workers, and Mr. J. de Laclémandière (France) set out his views on information and the 'law of least effort'. The afternoon was left free for visits and other activities and in the evening a reception was held by H.M. Government in the unique setting of the King's Library, British Museum, when Lord and Lady Hailsham received the guests.

The final public session and the official closure of the conference took place on the afternoon of September 15, with Dr. Alexander King, president of the Federation, in the chair. The General Assembly this year had been attended by representatives of twenty-four member countries and by observers from several other regions. Dr. King welcomed the presence of Miss Hilda S. Lindstedt, formerly librarian of the Royal Technical University, Stockholm, and now an honorary member of the Federation. Dr. King went on to say that the decision to break away from tradition and to hold the 1960 meetings at Rio de Janeiro had had important consequences for the International Federation for Documentation, including some changes in constitutional procedure. In future, presidents would be elected a year in advance of the date on which they would take office and retiring presidents would serve an additional year as members of the Executive Bureau; all this made for continuity.

Dr. King announced that Dr. Burton W. Adkinson would succeed him as president in 1962. Vice-presidents were working appointments chosen on a regional basis. The vice-presidents for Western Europe were now Miss Barbara Kyle (United Kingdom), Dr. Z. Paulo (Portugal), Dr. W. Schurmeyer (Germany) and Prof. E. Wegelius (Finland). East European vice-presidents were Dr. L. Janzky (Hungary) and Prof. A. I. Mikhailov (U.S.S.R.). Dr. S. R. Ranganathan (India) had retired and his place had not yet been filled, but Far Eastern representation was in the capable hands of Prof. H. Ootuka (Japan). For the Americas, Mr. K. F. Heumann (United States) would replace Dr. Burton Adkinson, now president-elect, and Prof. Lydia Sambaquy (Brazil) would continue in office.

Turning to future conferences, Dr. King announced that the meeting for 1962 would be at The Hague. It was hoped that a meeting in one of the Asian countries could be arranged for 1963 or 1964, and the United States had issued an invitation to hold the next unrestricted conference in Washington in 1965.

Dr. King then dealt with the committees, the secretariats of which were now each located in different member countries. Mr. E. Ohman (Sweden) had been appointed the first full-time officer of the Universal Decimal Classification at The Hague. The training of personnel for carrying out documentation was being actively investigated under Prof. Majewski at Warsaw, a newly constituted committee to consider the theory of classification would be based in Denmark, responsibility for linguistic problems had been accepted by the Netherlands, and mechanized documentation was the special concern of the German

section. A new *ad hoc* Committee had been set up to give an impartial study to the future of the Universal Decimal Classification.

For the closing moments of the conference Dr. Burton Adkinson took over the chair and voiced the thanks of the delegates to the representatives of the host country, and in particular to Aslib, for the organization of the conference and for the accompanying programme of visits and entertainment.

E. M. R. Dittmas

X-RAY MICRO-ANALYSIS

List 177/2, recently issued by the Cambridge Instrument Co., Ltd., is an illustrated booklet* giving a comprehensive description of the Company's 'Microscan' X-ray analyser which is now in production. The technique of X-ray micro-analysis consists of focusing an electron beam, normally accelerated from 0 to 50 kV., on to a region of the surface of the specimen, approximately 1μ in diameter and rather less in depth, and analysing spectroscopically the resulting X-rays excited from the specimen. R. Castaing, in 1951, published details of the first practical instrument that could be described as an 'emission' micro-analyser, but Castaing's instrument, in common with similar instruments developed independently by others, used a static electron probe; it was thus limited to carrying out only point-by-point micro-analyses on parts of the sample surface previously selected under an optical microscope. The Cambridge instrument incorporates a deflexion system to control the electron probe, and in addition to static point-to-point micro-analyses it can also be used for scanning a maximum area of about $\frac{1}{2} \times \frac{1}{2}$ mm.^{2} on the sample surface. It is based on the instrument originally designed by Dr. D. A. Melford and colleagues at the Tube Investments Research Laboratories in collaboration with Dr. V. E. Cosslett and Dr. P. Duncumb, who were responsible for the prototype developed at the Cavendish Laboratory, University of Cambridge.

In the booklet the arrangement, constructional details and functioning of the analyser are clearly set out, together with photographs of parts of the instrument and of results obtained with the instrument. The analyser consists of four separately standing sections—the electrical supply unit, the main console, and three racks occupied mainly by scanning, counting and display electronic equipment, respectively. In the main console are housed the vacuum system consisting principally of a 2-in. oil diffusion pump backed by a rotary pump; the electron-optical system; the specimen chamber on which the electron-optical system is mounted and which is designed to allow from outside the maximum possible manipulation of the specimen which is itself under high vacuum. Sixteen positions are provided on the table and the table can be rotated to bring any one of the positions under either the electron beam or the optical microscope; the binocular optical microscope for conventional optical examination of the specimen for comparison with the electron image; and the semi-focusing X-ray spectrometer of a special and simplified design, containing three curved, rotatable, lithium fluoride crystals and two interchangeable detectors, one a sealed-off proportional counter for the detection of hard radiations and the other a flow proportional counter for soft radiations.

The technique of X-ray micro-analysis has so far been successfully applied to investigating the microstructure of both metals and minerals, and although the 'Microscan' was designed primarily for metallurgical work it can be used for substances other than metals. By providing both X-ray and electron pictures of small surface regions, the analyser is invaluable as a metallurgical tool, and the examples of its application to the examination of various alloys and inclusions in steel which are discussed and illustrated in the booklet show clearly its advantages. Its main limitation, in common with other similar instruments, is its inability at present to detect the soft X-rays from specimens consisting of elements of atomic number less than twelve.

* *The Microscan X-ray Analyser*, Pp. 32 (London: Cambridge Instrument Co., Ltd., 1961).

DISTRIBUTION OF INTENSITY AMONG VARIOUS ULTRASONIC DIFFRACTION ORDERS

By Dr. C. RAGHUPATHI RAO
Department of Physics, Nizam College, Hyderabad

The generalized theory of Raman and Nath gives very complicated expressions which, with certain restrictions, have been modified by Nath[1] to get closed expressions for intensities of ultrasonic diffraction orders. The intensity of the supersonic field is assumed to be not strong enough to excite the second and higher orders and solving the difference-differential equation obtained in the generalized theory, the following expressions for the intensities of the first and central zero orders have been obtained for normal incidence:

$$I_1 = \frac{4}{\rho^2 + 8} \sin^2 \left\{ \frac{\sqrt{\rho^2 + 8}}{4} \xi \right\}$$

$$I_0 = 1 - \frac{8}{\rho^2 + 8} \sin^2 \left\{ \frac{\sqrt{\rho^2 + 8}}{4} \xi \right\} \qquad (1)$$

where $\rho = \frac{\lambda^2}{\omega \phi_0 \lambda^{*2}}$ and $\xi = \frac{2\pi\omega L}{\lambda}$

This expression clearly reveals that the intensity of the zero order I_0 on diffraction is less than unity by a value of $2 \times I_1$ where I_1 is the intensity of the first order of diffraction. The intensity of the zero order is shared by the two first orders of diffraction equally on either side of the central order. If the individual intensities of various orders, I_0, I_{+1} and I_{-1} could be measured accurately, then it can be expected, according to expression (1), that the sum of the three intensities, that is $(I_0 + I_{+1} + I_{-1})$, should be equal to the original slit intensity I of the zero order without diffraction. With the view of verifying the above statement, intensities of the various diffraction orders were measured with a sensitive photomultiplier tube and the results obtained are presented here.

It is very important to note that only the first orders of diffraction are excited, since expression (1) ignores the intensity of higher orders. Further, when second and higher orders are excited, multiple diffraction effects set in and these are carefully avoided in the present experiments using very low acoustical power.

Table 1. Intensity Distribution among Various Orders
(f = 3·8 Mc./s.—normal incidence)

Liquid	I_0/I (per cent)	I_{+1}/I (per cent)	I_{-1}/I (per cent)	Aggregate intensity as percentage of incident intensity
Water	93·9	1·1	1·1	96·1
Acetone	96·6	1·6	1·6	99·8
Methyl alcohol	98·0	0·6	0·6	99·2
Toluene	97·2	0·7	0·7	98·6

A progressive wave in water at 3·8 Mc./s. is set up and the intensities of I_0, I_{+1} and I_{-1} on diffraction and the intensity I of zero order without diffraction are measured with the photo-tube. The intensity of the diffraction order expressed as a percentage of the original slit intensity of the zero order is tabulated in Table 1 using different liquids. Table 1 clearly shows that the aggregate intensity of various diffraction orders compares very well with the intensity of the original incident light in the zero order. These experimental results are in good agreement with expression (1).

The second aspect of interest is to study the distribution of intensity among various ultrasonic diffraction orders with obliquity of the sound wave-front. Raman and Nath[2], starting from very simple elementary considerations, have shown that diffraction effects disappear periodically, whenever the obliquity Φ of the sound wave-front from the position of normal incidence is equal to $\tan^{-1}\frac{n\lambda^*}{L}$, where $n \neq 0$ is an integer, λ^* is the sound wave-length in the medium and L is the length of the ultrasonic path traversed by the light beam. The expression for the relative intensities is given by: $\frac{J_m^2(V)}{J_{n2}(V)}$ where:

$$V = V_0 \sec\Phi \frac{\sin\left(\frac{\pi L}{\lambda^*}\tan\Phi\right)}{\pi L/\lambda^* \tan\Phi} \qquad (2)$$

and $$V_0 = \frac{2\pi\omega L}{\lambda}$$

The behaviour of the above expression explains very well the variation of intensity with Φ among various diffraction orders and it is interesting to study this phenomenon with the photo-multiplier.

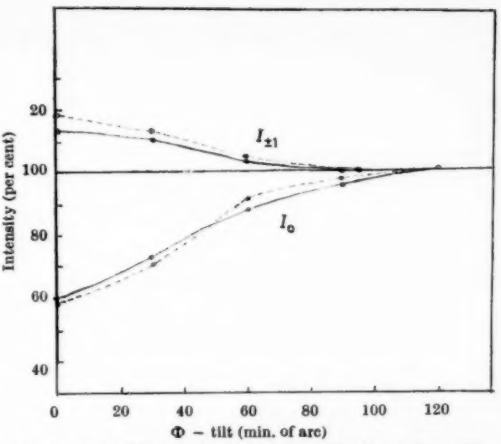

Fig. 1. Variation of intensity of the diffraction orders with obliquity of the sound wave-front
——, Experiment; – – –, theory; f = 3·8 Mc./s.; L = 1·5 cm.

As before, a progressive wave in water at 3·8 Mc./s. is employed and, using moderate power, only the first orders of diffraction on either side of the zero order are excited. The intensities of I_0, I_{+1} and I_{-1} are recorded with the photo-tube for various angles of obliquity Φ in steps of 30′. The value of the parameter V is calculated for the angles of tilt Φ chosen using the expression (2) and the corresponding intensities are evaluated using the Bessel functions $\frac{J_m^2(V)}{J_n^2(V)}$. The value of L used here is 1·5 cm. The theoretical results evaluated in this way and the experimental ones obtained by the photo-tube for I_0 and $I_{\pm1}$ are plotted in Fig. 1. It can be seen that the agreement between the two curves is very satisfactory, showing the validity of expression (2). The intensity $I_{\pm1}$ becomes zero at 95′, as can be seen in Fig. 1, and this value compares very well with the angle of 92′ computed by $\tan^{-1}\frac{\lambda^*}{L}$ for the disappearance of the first order.

Expression (2) is very important in that it can predict the poor intensities of the very high frequency diffraction orders observed by several investigators[3,4]. It is well known that at moderately high frequencies, the sound wave-front is to be tilted with respect to the light beam to make the first-order diffraction line appear and therefore the tilt angle Φ is to be taken into account in estimating the intensity of the high frequency diffraction orders. The expression (2) can be written as: $V = V_0 \sec\Phi \frac{\sin t}{t}$

where $t = \frac{\pi L \tan\Phi}{\lambda^*}$.

The parameter V_0 in the case of normal incidence is given by $V_0 = \frac{2\pi\omega L}{\lambda}$ and does not include λ^*. But the moment the light beam is tilted, as in the case of high frequencies, the parameter V includes not only V_0 but also λ^*, the sound wave-length in the medium. It is important to see how Φ affects V. The value of Φ involved experimentally is generally small, namely,

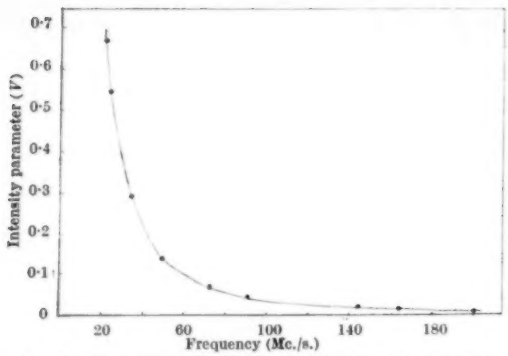

Fig. 2. Behaviour of the intensity parameter with increasing frequencies. $L = 1{\cdot}5$ cm.

$< 2°$, and therefore one can approximate $\tan \Phi$ and $\sin \Phi$ to Φ and write $t = \frac{\pi L \Phi}{\lambda^*}$. But $\sin t$ is never equal to t under the experimental conditions since t involves $\frac{\pi L}{\lambda^*}$, which is very large. At high frequencies $\frac{L}{\lambda^*}$ tends to increase as λ^* decreases with increasing frequencies; t therefore assumes large values making $\sin t$ approach its maximum value unity at very high frequencies. Under these valid approximations, the parameter V can be written as $V = V_0/\pi L \left(\frac{\lambda^*}{\Phi}\right)$ and is directly proportional to $\left(\frac{\lambda^*}{\Phi}\right)$. At high frequencies, Φ approaches the Bragg angle[5] to bring the diffraction order into the field of view and λ^* decreases, with the result the ratio $\frac{\lambda^*}{\Phi}$ tends always to decrease with increasing frequencies. Therefore the intensity parameter V goes on diminishing with high frequencies as shown in Fig. 2, thus giving a natural explanation to the poor intensities observed in high-frequency ultrasonic diffraction phenomena.

[1] Nath, N. S. Nagendra, *Proc. Ind. Acad. Sci.*, **8A**, 499 (1938).
[2] Raman, C. V., and Nath, Nagendra, *Proc. Ind. Acad. Sci.*, **2**, 413 (1935).
[3] Rao, B. R., thesis, Andhra University (1948).
[4] Rao, C. R., thesis, Osmania University (1953).
[5] Bhagavantam, S., and Rao, B. R., *Nature*, **161**, 927 (1948).

EIGHT-CO-ORDINATE DIARSINE COMPLEXES OF QUADRIVALENT METAL HALIDES

By Dr. R. J. H. CLARK, Dr. JACK LEWIS, Prof. R. S. NYHOLM, F.R.S., Dr. PETER PAULING and Dr. G. B. ROBERTSON

William Ramsay and Ralph Forster Laboratories, University College, Gower Street, London, W.C.1

As part of a systematic study of the ditertiary arsine complexes of transition metal halides, we have been investigating the behaviour of titanium, zirconium and hafnium tetrachloride with the chelate group *o*-phenylene*bis*dimethylarsine[1] (diarsine or *D* in the following account)[1]. The ditertiary arsine complexes of metals in the first half of the transition series have been little investigated. One reason for this is undoubtedly the great interest in transition metals which have potential double-bonding capacity such as nickel, palladium and platinum. In addition, the metals at the beginning of each transition series were thought to co-ordinate with the more electronegative donor atoms such as oxygen rather than with arsenic. Provided that one uses a non-hydroxylic solvent as the reaction medium, however, these transition metals will co-ordinate with the diarsine.

We have prepared a mono-chelate derivative of titanium tetrachloride with the formula $TiCl_4$.diarsine by the reaction of titanium tetrachloride with the diarsine in 1 : 1 molar ratio in carbon tetrachloride solution[2]. The composition of this compound has been confirmed by analysis and some of its properties are given in Table 1. It is a non-conductor in nitrobenzene solution; it is considered to be a typical octahedral non-electrolyte but it is not iso-structural with $SnCl_4$.diarsine originally described by Allison and Mann[3].

From the reactions between the diarsine and zirconium tetrachloride and hafnium tetrachloride in acetone, we have isolated only the *bis*-diarsine compounds $ZrCl_4$.2diarsine and $HfCl_4$.2diarsine. The corresponding *bis*-diarsine titanium tetrachloride complex $TiCl_4$.2diarsine can be prepared by treating $TiCl_4$.diarsine with excess diarsine or by a curious reaction between titanium trichloride and the diarsine in glacial acetic acid[2]. The reaction of vanadium tetrachloride with the diarsine in carbon tetrachloride solution gives VCl_4.2diarsine. This compound is paramagnetic, with one unpaired electron, as expected. Some properties of these four *bis*-diarsine complexes are shown in Table 1. They are iso-structural as shown by X-ray powder photography and have nearly identical infra-red spectra.

The *bis*-diarsine titanium complex, which is slightly soluble in nitrobenzene, has been more fully investigated than the other derivatives because they have much lower solubilities in all solvents tried. In nitrobenzene, $TiCl_4$.2diarsine forms a non-conducting solution; this implies that in solution either: (1) the complex involves an eight-co-ordinated titanium atom, the diarsine behaving as a chelate group; or

Table 1. Properties of Ditertiary Arsine Complexes

Compound	Colour	Melting point in deg. (decompr.)	Conductivity in nitrobenzene		
			Concentration (mol.cm.$^{-3}$)	Λ_∞ (cm.^{2}ohm^{-1} mol.$^{-1}$)	μ_{eff} (B.M.)
$TiCl_4.D$	yellow-orange	210	$5{\cdot}0 \times 10^{-3}$	0·5	0·0
$TiCl_4.2D$*	orange-red	174	$2{\cdot}3 \times 10^{-3}$	0·9	0·0
$VCl_4.2D$*	orange	134	—	—	1·8
$ZrCl_4.2D$*	white	290	—	—	0·0
$HfCl_4.2D$*	very pale pink	284	—	—	0·0

* Isostructural D = diarsine

Satisfactory analytical results for carbon, hydrogen, chloride, arsenic and metal have been obtained for these compounds.

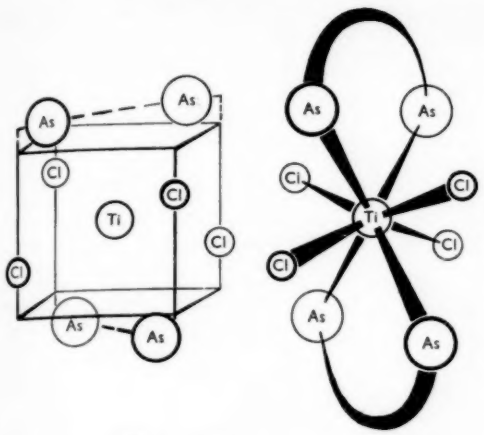

Fig. 1. (a) Drawing showing the relationship between the co-ordination polyhedron and a cube; (b) a perspective view of $TiCl_4$.2diarsine in which the phenyl rings are represented diagrammatically

(2) the complex dissociates, forming, for example, one mole of $TiCl_4$.diarsine and one mole of free diarsine. (We consider that the possibility that the titanium atom is six-co-ordinate with the diarsine behaving as a monodentate group is excluded by the infra-red spectrum.)

The diffuse ultra-violet and visible spectra of the solid $TiCl_4$.diarsine and $TiCl_4$.2diarsine have been recorded. The charge transfer band of the former occurs at 4650 Å. and of the latter at 5100 Å. Both compounds, however, show the same spectrum in polar solvents ($\lambda_{max.} \sim 4600$ Å.) and in benzene ($\lambda_{max.} = 4480$ Å.). This suggests that $TiCl_4$.2diarsine dissolves by dissociation into $TiCl_4$.diarsine and free diarsine. Both compounds can be recrystallized from benzene and nitrobenzene solutions, but the *bis*-diarsine complex is less soluble, presumably because of the mass action effect of the liberated diarsine.

From the infra-red spectrum and an X-ray diffraction investigation of $TiCl_4$.2diarsine, we have shown that in the solid state both diarsine molecules are co-ordinated to the titanium atom. The intense infra-red bands at 884, 846 and 741 cm.$^{-1}$ of the free diarsine are not present in the compound, but corresponding bands some 20 cm^{-1}. higher in frequency are present. This shift is characteristic of co-ordinated diarsine.

The structure of the solid $TiCl_4$.2diarsine has been studied by single-crystal X-ray diffraction methods. The point symmetry of the titanium atoms is $\bar{4}2m$, with all the atoms in the molecule except the methyl groups lying on mirror planes. The co-ordination polyhedron around the titanium atom is a dodecahedron with eight vertices consisting of four equivalent arsenic atoms and four equivalent chlorine atoms. The arsenic atoms are 2·71 ± 0·02 Å. from the titanium atom and are at an angle of 36° to the tetragonal axis. The chlorine atoms are 2·46 ± 0·02 Å. from the titanium atom and are at an angle of 73° to the tetragonal axis. So far as we know, these are the first examples of eight-co-ordination of atoms in the first long period of the Periodic Table.

The molecular symmetry of these compounds is the same as that reported for the complex ion $[Mo(CN)_8]^{4-}$ (ref. 4). For this symmetry a set of hybrid bond orbitals determined by Racah[5] from d^4sp^3 orbitals has one group of four with maximum strength 2·968 at an angle of 72° 47′ to the tetragonal axis and another group of four with maximum strength 2·995 at an angle of 34° 33′ to the tetragonal axis.

The direction of the titanium–chlorine bond coincides with that of the first group of these hybrid bond orbitals. The angle between the direction of the titanium–arsenic bond and the tetragonal axis (36°), however, is 2° larger than that of the corresponding maximum strength hybrid orbital, while the neighbouring carbon′–carbon–arsenic angle (117°) is 3° smaller than the ideal trigonal angle of 120°. While barely significant, this suggests that the stability of the molecule is sensitive to the titanium–arsenic distance, and that the angular distortion necessary to obtain this distance is correspondingly distributed between these two angles.

An investigation of the reactions of diarsine with other tetrachlorides and with other halides is in progress. Further details of this work will be published elsewhere in full.

We are indebted to British Titan Products, Ltd. (R. J. H. C.), and to the United States Department of the Army through its European Research Office (G. B. R.) for financial support, and to O. S. Mills and J. S. Rollett for the use of their computer programmes.

[1] Chatt, J., and Mann., F. G., *J. Chem. Soc.*, 610 (1939).
[2] Sutton, G. J., *Austral. J. Chem.*, **12**, 122 (1959).
[3] Allison, J. A. C., and Mann, F. G., *J. Chem. Soc.*, 2915 (1949).
[4] Hoard, J. L., and Nordsieck, H. H., *J. Amer. Chem. Soc.*, **61**, 2853 (1939).
[5] Racah, G., *J. Chem. Phys.*, **11**, 214 (1943).

UNSTABLE FLOW OF MOLTEN POLYMERS

By J. J. BENBOW, R. V. CHARLEY and P. LAMB

Plastics Division, Imperial Chemical Industries, Ltd., Welwyn Garden City

WHEN molten polymers are extruded at low stresses through dies of circular cross-section, the extrudates are smooth and cylindrical. At higher stresses the surface may be marred by two defects. The first is known as mattness or in its more severe form as 'shark-skin', which consists of ridges at a separation usually small compared with the diameter of the extrudate; it tends to occur at a constant value of the average velocity and is attributed to a stick-slip motion at the die exit[1]. The second is on a larger scale, and the extrudates may take a helical or an irregular, distorted form. This second defect, with which this article is concerned, has been attributed[2] to 'melt fracture' within the polymer. The term fracture implies that the ultimate cause of the distorted extrudates lies in the failure of inter- or

intra-molecular bonds within the melt. The evidence to be presented suggests the origin of the defect as being a stick-slip phenomenon at the die wall.

In extrudates of polytetrafluoroethylene and polyvinyl chloride there is clear evidence of macroscopic fracture, with the formation of free surfaces, but it has not been demonstrated that these arise before the material enters the die; they could be produced at the exit. In less viscous polymers such as 'Diakon', polythene and polypropylene, fracture is not obvious in the extrudate, but is sometimes inferred from the 'breakage' during extrusion of a central coloured filament embedded in colourless material; in experiments with transparent apparatus the apparent breakage is seen to occur before the material enters the die.

As part of an investigation into the causes of distortion in extrudates, we have carried out experiments to examine the possibility of slipping at the die wall. Clegg[3] considered the possibility of a stick-slip mechanism being responsible for unstable flow, but his experiments showed that changes in the roughness of the die surface had little effect on the flow of polythene. However, it seems likely that the polymer properties rather than the roughness of the surface are usually predominant in determining the onset of slippage, since on a molecular scale all surfaces are rough. In our experiments we measured the distribution of velocity in the die at extrusion-rates above and below the point of instability. The movement of a coloured disk of poly(dimethyl siloxane) gum was determined from ciné films of its motion in the die after the sudden application of the appropriate shear rate. Poly(dimethyl siloxane) gums were used because their flow behaviour at room temperature resembles that of molten polymers at much higher temperatures[1]. Working at room temperature avoids elaborate temperature control equipment and makes possible the use of easily fabricated transparent 'Perspex' dies.

The results are shown in Fig. 1, where the relative displacements are shown at equal time-intervals after an arbitrary zero chosen when the velocity of the extrudate reached a constant value. It is evident that slipping is indicated in the two high stress measurements (*a*), (*b*), where at low stresses the profile (*c*) is very similar to that predicted (*d*) from the measured shear stress–shear rate relation.

The displacement profiles in (*a*) and (*b*) suggest that 'plug' flow is occurring, but similar experiments, using a less viscous silicone gum, show that when slipping takes place there is still, as would be expected for such material, a wide distribution of velocities within the melt. This probably explains why it is difficult to detect slipping in low-viscosity polymers since the large range of velocities in the melt mask the relatively small difference in velocity between the die wall and the material in contact with it. Conversely, it is easier to detect slippage in highly viscous liquids.

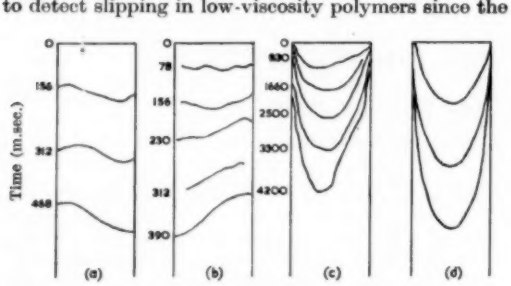

Fig. 1. Displacement profiles of poly(dimethyl siloxane) at high and low stresses. *a*, stress = $1{\cdot}2 \times 10^6$ dynes/cm.^{2}; *b*, stress = $1{\cdot}2 \times 10^5$ dynes/cm.^{2}; *c*, stress = 6×10^5 dynes/cm.^{2}; *d*, profile calculated from measured stress–shear-rate relationship

Because the stress was applied suddenly and the measurements necessarily ended when the disk was extruded, it may be argued that the experiments are too transient in character and do not correspond to steady-state extrusion. However, this criticism applies equally to the experiments above and below the point of instability, and the difference in behaviour remains valid. To establish this point more convincingly, additional qualitative experiments were carried out in which coloured material was injected at the die wall under steady-state conditions and these again showed no slip at a low stress, but appreciable slip when the flow was unstable. Similar experiments with highly viscous molten polythene extruded through a glass die also showed that slipping was taking place when the stress was above that at which instability began. Our results show that when molten polymers are extruded at sufficiently high stresses the velocity at the die wall is not zero. Since the stress at which slipping occurs is near the stress at which the results of unstable flow in the die can be detected in the extrudate, this suggests that the two effects are connected and that the unstable flow may be due to a stick-slip mechanism.

The typical forms which non-cylindrical distorted extrudates take in molten polymers can also be qualitatively explained in terms of a stick-slip mechanism; helical extrudates found with many polymers could be caused by a precession of the point of slipping or the point of maximum slip around the circumference of the die, and the defect known as 'kneeing' in polypropylene could be caused by slippage taking place alternately at opposite ends of a diameter.

There is much indirect evidence which supports the stick-slip hypothesis. Experiments in which a metal plate is pulled across the surface of a pool of low-viscosity (10^4 poises) siloxane fluid show that at high speeds even a weighted slider will slip. When polythene is extruded it has been shown[4] that the 'swelling ratio' passes through a maximum at or below the point of instability; the decline from the maximum when instability sets in could be due to the storage of less elastic energy in the extrudate when slipping occurs. Since the stress is a maximum at the die wall and the onset of instability occurs at a critical stress, it is reasonable to expect the cause of the instability to be at the wall rather than in the polymer. The effects of slipping are more easily observed with highly viscous polymers, so that any differences in the amount of slip which takes place when using dies of different composition will also be more easily detected with highly viscous polymers. Experiments with a very viscous polythene melt suggest that, at high stresses, the stress for a given output-rate is slightly less through a polytetrafluoroethylene than through a steel die of the same dimensions.

The facts presented here seem to us strong evidence that unstable flow of molten polymers is associated with slipping at the die wall, and we further suggest that instability may be the result of a stick-slip mechanism.

[1] Benbow, J. J., Brown, R. N., and Howells, E. R., *Coll. Inter. Rheologie, Paris* (1960).
[2] Tordella, J. P., *J. App. Phys.*, **27**, 454 (1956).
[3] Clegg, P. L., *Rheology of Elastomers*, 174 (Pergamon Press, London, 1958).
[4] Beynon, D. L. T., and Glyde, B. S., *British Plastics*, 414 (1960).

SOMATIC CHROMOSOMES OF THE GORILLA

By

J. L. HAMERTON
Pædiatric Research Unit, Guy's Hospital Medical School, London, S.E.1

M. FRACCARO
Medical Research Council Population Genetics Research Unit, Headington, Oxford

L. DE CARLI and FIORELLA NUZZO
Istituto di Genetica, Università, Pavia

H. P. KLINGER and LOTTE HULLIGER
Anatomisches Institut der Universität, Basel

ANGELA TAYLOR
Hospital for Sick Children, Great Ormond Street, London, W.C.1

AND

E. M. LANG
Zoologischer Garten, Basel

ACCURATE knowledge of chromosome number, morphology and behaviour in mitosis and meiosis has often assisted genetical and evolutionary analysis at the species-level.

Recent technical advances have made possible a more accurate study of chromosomes in several species of mammals, including man[1]. By using *in vitro* cultures of biopsy specimens of skin[2,3] and peripheral blood[4], precise delineation of somatic chromosomes may be obtained. This involves little discomfort and no danger to the animals. As a direct result of these developments, the chromosome number of some twenty-five species and sub-species of primates has been recently determined[5]. The anthropoid apes, however, have thus far been relatively neglected, mainly because of the difficulties of obtaining suitable material. [See also p. 285 of this issue.]

The diploid chromosome number of the chimpanzee was determined as 48 by Yeager *et al.*[6], who used testis material. This was recently confirmed by Young *et al.*[7] in preparations from the bone-marrow of nine animals, but a detailed analysis of the karyotype is not as yet available.

We describe here the chromosomes of the gorilla and attempt a comparison with the human chromosome complement, of which several analyses have been published[8].

Two gorillas, one of each sex, kept at the Basle Zoological Gardens, were made available for examination while some of us were on the teaching staff of the World Health Organization international laboratory course in methods of human cell culture and cytology at the Anatomical Institute of the University of Basle. Both gorillas are of the Lowland sub-species (*Gorilla gorilla gorilla*, Savage and Wyeman). The male, which was about nine months old at the time of biopsy, was captured in the Cameroons in March 1960. The female was born in the Basle Zoological Gardens on the night of September 22–23, 1959. 8 ml. of blood were drawn from an arm vein of the male and cultures were prepared by a technique similar to that described for human leucocytes[4].

A skin biopsy was taken from the female and cultured by a plasma clot technique in the presence of a fluid medium. Cells from both animals were pre-treated with 'Colcemid' (Ciba, desacetylmethylcolchicine) and hypotonic solution, fixed and afterwards stained with acetic orcein or unna blue. Chromosome counts were performed directly on preparations from both primary and (with the skin) transferred cultures. Karyotype delineations and measurements of the relative lengths of individual chromosomes were obtained from enlarged photomicrographs. Absolute measurements were made with a micrometric ocular.

Sexual dimorphism of interphase nuclei. Smears of oral mucosa cells were obtained from both animals and stained with thionin[9]. 42 per cent of the nuclei in the female possessed a characteristic chromatin mass lying adjacent to the nuclear membrane (Fig. 1

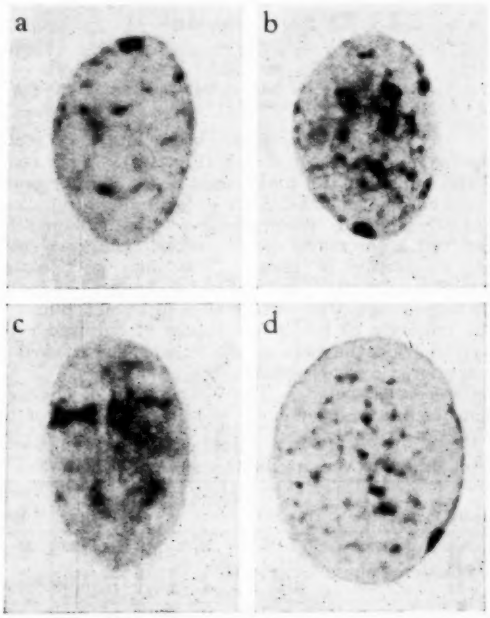

Fig. 1. Nuclei of buccal mucosa of the female gorilla (*a*, *b*, *c*) and of the human female (*d*) for comparison

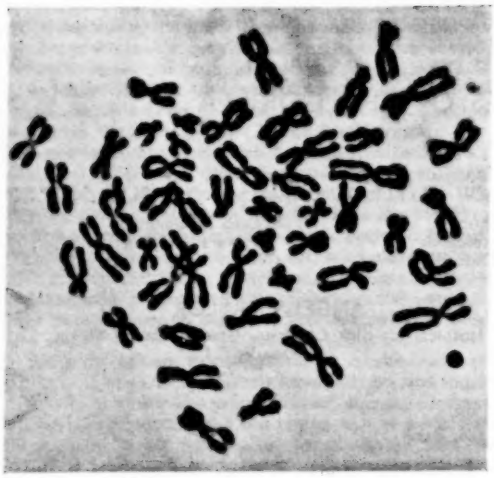

Fig. 2. Mitotic metaphase in cultured cell from peripheral blood of the male gorilla. Acetic orcein. Phase contrast. Photographic enlargement × 1,600

Fig. 3. Karyotype of the male gorilla. Constructed with the chromosomes of Fig. 2

a, *b*, *c*). This usually appears larger and more rectangular in shape than in man (Fig. 1 *d*). 4 per cent of the nuclei in the male contained a similar peripheral chromatin mass. A variable number of smaller, centrally placed chromocentres were frequently observed in the nuclei of both sexes. These are seen less frequently in man. The fine, particulate, nucleolar-associated chromatin was observed in about the same amount as usually seen in human nuclei[10]. The majority of interphase nuclei of cultured cells derived from the skin of the female also showed a characteristic sex-chromatin body.

Chromosome counts. The distribution of chromosome counts independently obtained by four of us is set out in Table 1.

Table 1. Chromosome Counts in the Gorilla

Individual	Type of culture	Observer	Chromosome No. 45	46	47	48	>48	Total
Male	Blood	J. L. H.	2		2	28	2*	34
Female	Skin	L. DC., F. N.	1	2	3	22	—	28
		M. F.	1	3	1	46	2*	53
	Total		4	5	6	96	4*	115

* Polyploid cells.

Table 2. Somatic Chromosomes of the Gorilla

Group 1–3. Long chromosomes with approximately median centromeres. It is difficult to distinguish them from each other. The *X*-chromosome is similar to these but easily separated by its length. It is the longest chromosome of the complement.

Group 4–12. Medium-sized chromosomes with median or sub-median centromeres. There is little difference in length between individual pairs in this group, and the chromosomes are difficult, if not impossible, to distinguish one from another by comparative measurements. Chromosome 12 can be identified by the presence of a prominent secondary constriction in its long arm, close to the centromere.

Group 13–18. Medium-sized acrocentric chromosomes. Chromosomes 13, 14, 15 bear satellites, chromosome 16 apparently does not. Chromosomes 17 and 18 are shorter than the other chromosomes in this group. Their short arms bear prominent satellites.

Group 19–21. Medium to short chromosomes with median centromeres. These can usually be distinguished from each other and from chromosomes of other groups by their length and centromere position.

Group 22–23. Short acrocentric chromosomes. Chromosome 22 bears prominent satellites, chromosome 23 has slightly smaller ones. The *Y*-chromosome is similar to these chromosomes, but generally appears more metacentric.

The diploid chromosome number is 48. The counts of less than 48 chromosomes are probably due to loss of chromosomes during the technical procedures. A few of these cells were analysed and no consistent pattern of chromosome loss was found. The four cells with chromosome counts greater than 48 were probably polyploid, and two of them had exact counts of 96 chromosomes.

Karyotype analysis. A summary of the chromosome morphology of the gorilla is given in Table 2,

Fig. 4. Mitotic metaphase in cultured skin cell of the female gorilla. Acetic orcein. Phase contrast. Photographic enlargement × 1,600

Fig. 5. Karyotype of the female gorilla. Constructed with the chromosomes of Fig. 4

Table 3. MEANS AND RANGE OF VARIATION (IN BRACKETS) OF THE ABSOLUTE LENGTH (IN μ) OF THE LONGEST AND SHORTEST CHROMOSOMES OF THE GORILLA AND HUMAN COMPLEMENT
The estimates are based on measurements performed on 10 cells (treated with colchicine) for each type of tissue

Chromosome measured	Blood		Skin	
	Gorilla	Man	Gorilla	Man
Longest	5·3 (3·6–6·3)	5·6 (4·5–8·0)	6·3 (4·5–8·1)	6·4 (5·9–7·2)
Shortest	1·6 (1·0–1·9)	1·6 (0·9–1·8)	2·2 (1·4–2·7)	1·6 (1·4–1·8)

and representative metaphase plates and karyotypes in Figs. 2–5. The complement has been divided into five groups and the chromosomes numbered so far as possible in order of descending length. The presence of secondary constrictions or, in the case of acrocentric chromosomes, of satellites, has aided the identification of certain chromosomes. This system is based on the same criteria as the Denver standard system of nomenclature proposed for the human chromosome complement.

Idiograms of the chromosome sets of the gorilla and man, based on average relative measurements of the chromosomes of seven cells from the two gorillas and on the measurements of human chromosomes given in the Denver statement[11], are compared in Fig. 6. The major points of difference are:

(1) The *X*-chromosome of gorilla is metacentric and is the largest chromosome in the complement.

(2) Chromosome 12 has a prominent secondary constriction in its long arm; no counterpart of this chromosome is found in the human karyotype.

(3) Six pairs of medium-sized acrocentric chromosomes (group 13–18) are present in gorilla and only five pairs in man (groups 13–15, 17–18).

(4) The acrocentric chromosomes of group 13–18 have more definite short arms and more prominent satellites than the acrocentric chromosomes (groups 13–15, 17–18) in man. Satellites of chromosome 13 (gorilla) are particularly prominent.

While several of the longest autosomes of gorilla are relatively shorter than those of man, absolute measurements of comparable cells (Table 3) show that there is no significant difference between the absolute lengths of the longest and of the shortest chromosomes in the two species.

Discussion. The similarities between the karyotypes of man and gorilla provide further evidence of the close evolutionary relationship of the two species. Man has one acrocentric chromosome less than gorilla, although the number of metacentric chromosomes is the same (Fig. 6).

Differences in chromosome number may originate in three ways: by polyploidy, polysomy or structural change. Polyploidy is a source of karyotype evolution in plants[12], but whether it has played any part in the evolution of mammals remains uncertain[13,14]. Polysomy is now known to be a component of pathological chromosome variation in man[15] and the mouse[16]. Changes of the type first recorded by Robertson[17] in the Acrididae have been found in two species of mammals, and cause inter- and intra-specific variation in chromosome number with little or no loss of chromosome material[18].

One of the metacentric chromosomes of the gorilla (No. 12 in our system) has a prominent secondary constriction in its long arm. A similar characteristic autosome has been reported in the chromosome complements of all species of primates which have been examined[5], but not in man. The size and position of the secondary constriction are morphologically similar throughout the group, although the autosome which contains it may differ in length and arm ratio from species to species.

The significance of this chromosome region is uncertain and its absence in man may be explained in several ways. A single reciprocal translocation with one break near the centromere of one of the long acrocentric chromosomes, followed by loss of the centric fragment, is one way in which the change in number may have occurred. If the other break were to have been in or near the secondary constriction of chromosome 12, it would also conceivably explain the apparent disappearance of this region.

The *X*-chromosome of the gorilla is relatively longer than the human *X*-chromosome. This might be correlated with the apparent larger size of the sex-chromatin mass in the nuclei of the female gorilla. The increased number of centrally placed chromocentres observed in the nuclei of the buccal mucosa cells of the gorilla may be similarly related to the increased number of negatively heteropyknotic regions observed in the metaphase chromosomes. Further speculation about the mechanism of karyotype evolution in man and

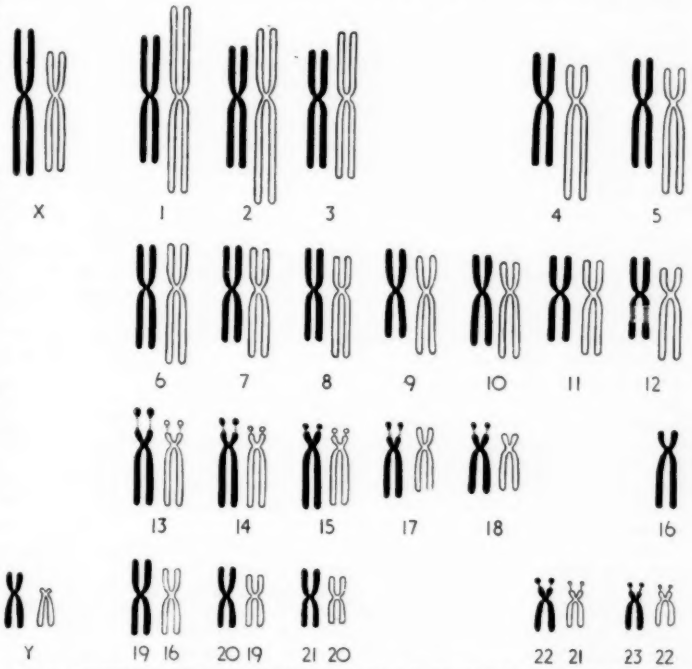

Fig. 6. Tentative comparison of the idiograms of gorilla (black) and man (outlined). The human chromosomes are numbered according to the Denver system (ref. 11)

the anthropoid apes must wait additional data. Now that simple biopsy techniques are available, institutions with collections of anthropoids will perhaps allow examination of the chromosomes of their charges. To quote the words of Darlington[14]: "Great institutions have been devoted to collecting, and preserving, describing and naming, the bony fragments of our ancestors and relatives, and great bodies of literature have been brought forth by their labours. Rightly so. Yet at the cost of a few hundred cells, and a few drops of blood, a few moments of discomfort to the animals, and a few hours of work to a cytologist, the genetic record of human evolution could be laid bare. It would, perhaps, be worth while".

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[1] Tjio, J. H., and Levan, A., *Hereditas*, **42**, 1 (1956).
[2] Harnden, D. G., *Brit. J. Exp. Path.*, **41**, 31 (1960).
[3] Fraccaro, M., Kaijser, K., and Lindsten, J., *Ann. Hum. Genet., Lond.*, **24**, 45 (1960).
[4] Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M., and Hungerford, D. A., *Exp. Cell Res.*, **20**, 613 (1960).
[5] Chu, E. H. Y., and Giles, N. H., *Amer. Nat.*, **91**, 273 (1957). Bender, M. A., and Mettler, L. E., *Science*, **128**, 186 (1958).
[6] Yeager, C. H., Painter, T. S., and Yerkes, R. M., *Science*, **91**, 74 (1940).
[7] Young, W. J., Merz, T., Ferguson-Smith, M., and Johnston, A. W., *Sciences*, **131**, 1672 (1960).
[8] Ford, C. E., Jacobs, P. A., and Lajtha, L. G., *Nature*, **181**, 1565 (1958). Tjio, J. H., and Puck, T. T., *Proc. U.S. Nat. Acad. Sci.*, **44**, 1229 (1958). Chu, E. H. Y., and Giles, N. H., *Amer. J. Hum. Genet.*, **11**, 63 (1959). Levan, A., and Hsu, T. C., *Hereditas*, **45**, 665 (1959). Fraccaro, M., and Lindsten, J., *Folia hered. path. Milano*, **9**, 185 (1960).
[9] Klinger, H. P., and Ludwig, K. S., *Stain Tech.*, **32**, 235 (1957).
[10] Klinger, H. P., and Schwarzacher, H. G., *J. Biophys. Biochem. Cytol.*, **8**, 345 (1960).
[11] Human Chromosome Study Group, Denver, *Ann. Hum. Genet., Lond.*, **24**, 319 (1960).
[12] Darlington, C. D., *Recent Advances in Cytology* (Churchill, London, 1937). Darlington, C. E., and Wylie, A. P., *Chromosome Atlas of Flowering Plants* (Allen and Unwin, London, 1946).
[13] White, M. J. D., *Animal Cytology and Evolution* (Camb. Univ. Press, 1954).
[14] Sachs, L., *Heredity*, **6**, 357 (1952). Darlington, C. D., *Nature*, **171**, 191 (1953).
[15] Ford, C. E., *Chromosomal Abnormality and Congenital Malformations*, Ciba Foundation Symposium on Congenital Malformations, 32 (Churchill, London, 1960).
[16] Cattanach, B. M., *Genet. Res., Camb.*, **2**, 156 (1961).
[17] Robertson, W. R. B., *J. Morph.*, **27**, 129 (1916).
[18] Wahrman, J., and Zahavi, A., *Nature*, **175**, 600 (1955). Sharman, G. B., *Nature*, **177**, 941 (1956). Ford, C. E., Hamerton, J. L., and Sharman, G. B., *Nature*, **180**, 392 (1957).

EFFECT OF GAMMA-RAYS ON THYMUS NUCLEOPROTEIN IN SOLUTION

By Dr. A. R. PEACOCKE and Dr. B. N. PRESTON
Department of Biochemistry, South Parks Road, Oxford

THERE have been many investigations into the action of ionizing radiations on deoxyribonucleic acid (DNA) and, from the point of view of macromolecular structure, the effects may be broadly classified as degradation into smaller fragments and denaturation, that is, loss of the double helical conformation. Since these substances occur *in vivo* only in combination with protein, earlier work[1-3] has been extended to an examination of the effects of γ-irradiation on the molecular weight and shape of the soluble deoxyribonucleohistone which may be obtained from calf thymus glands by extraction with water after a preliminary washing of the glands with versene and saline[4]. The method afforded dilute solutions of the nucleoprotein in 0·7 m*M* phosphate buffer (*p*H 6·8). The viscosities of these solutions, as measured in Ubbelohde viscometers, changed by less than 5 per cent during five days at 25° C.

The molecular weights of nucleoprotein were determined by the method of light-scattering (details in ref. 2) and had an average value of 22×10^6, with a maximum variation of 10 per cent from one independent extraction to another. Better reproducibility was frequently obtained, as in the particular preparations used in this work (Table 1). The stability and reproducibility of this material are thus confirmed. Analysis of these preparations (Mr. P. M. Bayley) showed that the N/P ratio was 3·70 in agreement with Doty and Zubay[4]. Nucleoprotein solutions were clarified from dust by centrifugation at 29,000 r.p.m. for 30 min. (Spinco Model *L*); no fractionation occurred during this centrifugation.

The solutions of nucleoprotein (about 0·02–0·09 per cent) in 0·7 m*M* phosphate buffer were irradiated by γ-rays from a cobalt-60 source, without exclusion of oxygen. Dosages (R) are stated as eV./phosphorus atom, where 1 eV./phosphorus atom is equivalent to 28·1 or 13·2 kilorads given to a 1 mgm./ml. solution of nucleic acid or nucleoprotein, respectively. After irradiation, the solutions were stored at 3° C. for at least 24 hr. and then used for light-scattering work. (This precaution proved to be unnecessary since no decrease in viscosity occurred during this period.) Aggregation of the nucleoprotein was observed at all dosages greater than 60 eV./phosphorus atom (up to 430 eV./phosphorus atom). Irradiation caused a decrease in viscosity, with no 'after-effect', and no increase in ultra-violet absorption at 259 mμ. This suggests that a decrease in molecular size occurred without denaturation of the nucleic acid of the complex.

The decrease in weight-average molecular weight (M_L) and in radius of gyration (ρ_θ), also derived from the light-scattering measurements, are shown in Fig. 1 and Table 1. At dosages of less than 18 eV./phosphorus atom, the relative decrease in molecular weight was a linear function of R, in contrast[2] to DNA, where it is dependent on R^2. This relation to R appeared to take a new direction above about 15 eV./phosphorus atom in the case of the most extensively studied preparation (D, conc. $= 4{\cdot}2 \times 10^{-4}$ gm./ml.). Moreover, the slope of the relation depended on the concentration of the nucleoprotein during the irradiation, even though R is expressed in eV./phosphorus atom, which should allow for the usual variation of an indirect effect with concentration. In all these respects, irradiation of the nucleoprotein differed from that of nucleic acid[1-3]. Another important difference was that, whereas ρ_θ of irradiated nucleic acid was[2] proportional to $M_L^{0.5}$, ρ_θ of irradiated nucleoprotein declined only very slowly as the molecular weight decreased: for example, to 70 per cent of its original value when the molecular weight

Table 1. Effect of γ-Irradiation on Calf Thymus Deoxyribonucleoprotein

Preparation :	$10^{-6} \times M_L$					ρ_g in Å.				
	B	*C*	*D*	*E*	*F*	*B*	*C*	*D*	*E*	*F*
Dosage *R* in eV./phosphorus atom										
0	22·5	22·7	22·2	21·8	22·0	2000	2100	2050	2200	2100
1·53			16·7					2100		
3·37		15·6					1800			
4·22			11·1					1700		
5·66		12·8					1950			
5·78			9·5					1800		
6·15				5·3					1600	
6·30	14·7					2000				
7·90			7·1					1600		
9·96			5·9					1600		
12·0			3·8					1300		
12·6	10·0					1800				
13·0			4·0					1300		
15·6			4·2					1300		
17·7				2·2					1750	
19·0	6·9					1800				
24·3			2·1					1300		
36·2			1·5					1300		

B, *C*, *D*, *E*, *F*, independent extractions of nucleoprotein. Concentrations of solutions as extracted and irradiated: *B*, $2{\cdot}3 \times 10^{-4}$ gm./ml.; *C*, $3{\cdot}77 \times 10^{-4}$ gm./ml.; *D*, $4{\cdot}2 \times 10^{-4}$ gm./ml.; *E*, $8{\cdot}77 \times 10^{-4}$ gm./ml. Solvent: 0·0007 *M* phosphate (*p*H, 6·8). M_L = mol. wt.; ρ_g = radius of gyration, both from light-scattering measurements.

had been reduced more than ten-fold, at $R = 24{\cdot}3$ and 36·2 eV./phosphorus atom. (In terms of a power relationship ρ_g is approximately proportional to $M_L^{0.3}$.) Clearly a process must be occurring whereby the molecular weight can be reduced without a comparable change in the extension of the molecular system primarily contributing to the observed ρ_g. The most likely explanation of this is that, on irradiation, the smaller protein units dissociate from the DNA, the molecular weight of which is unchanged and the extension of which is modified only relatively little. If this view were correct, the dissociation process should continue until the protein is fully dissociated when M_L should be approximately 4×10^6, taking the molecular weight of intact nucleic acid as 8×10^6. A value of $4{\cdot}5 \times 10^6$ for the mean molecular weight of the fully dissociated nucleoprotein system was observed when the solvent was 2·0 *M* sodium chloride. (This process of dissociation by salt has been the subject of separate work[5].) For this dissociated nucleoprotein, for non-irradiated and γ-irradiated deoxyribonucleic acid[2], and, in the present experiments, for γ-irradiated nucleoprotein of $M_L < 4 \times 10^6$ the values of the ratio $M_L^{1/2}/\rho_g$ were all approximately unity, in distinction from the value of 2·3 obtained with the original nucleoprotein. This suggests that when the mean molecular weight has fallen to less than 4×10^6, the shape is determined by nucleic acid free from protein. Beyond this point in the irradiation, the changes previously reported for nucleic acid[1-3] should be observed and denaturation should begin. Further experiments are needed to elucidate this point.

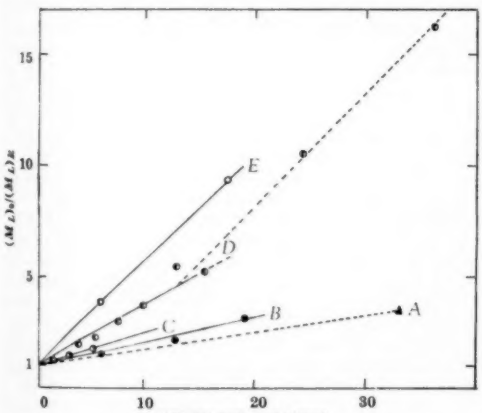

Fig. 1. Relative change in the molecular weight of thymus nucleoprotein caused by γ-irradiation.

Ordinate = (M_L before irradiation)/(M_L after dosage *R*). Concentration of nucleoprotein (independent preparations): *A* (▲), $1{\cdot}15 \times 10^{-4}$ gm./ml.; *B* (●), $2{\cdot}3 \times 10^{-4}$ gm./ml.; *C* (◑), $3{\cdot}77 \times 10^{-4}$ gm./ml.; *D* (◐), $4{\cdot}2 \times 10^{-4}$ gm./ml.; *E* (○), $8{\cdot}77 \times 10^{-4}$ gm./ml.

The first major effect of irradiation on the nucleoprotein complex therefore appears to be dissociation of the small protein molecules from the nucleic acid, which remains relatively intact until most of the protein is dissociated. This proposal is consistent with the earlier observation of Kaufmann *et al.*[6] that the decrease in viscosity of nucleoprotein solutions caused by X-rays did not involve depolymerization of the nucleic acid. Hems[7] has obtained evidence for rupture of the linkage between DNA and an acidic protein which is present in nucleoprotein prepared by the method of Kay *et al.*[8]. At what point in the dissociation nucleic acid itself begins to degrade and denature needs to be determined. The changes in M_L and ρ_g show that the nucleic acid must form a central core the extension of which is somewhat modified as the protein is removed, but not in proportion to the decline in its molecular weight. This is entirely in accordance with a structure for deoxyribonucleohistone (the protein is almost all histone in this preparation) which consists of a central core of double-helical deoxyribonucleic acid around which the histone chains are wound[9]. However, the possible role of any protein bridges must not be overlooked in more detailed work. The effect of changes in concentration (Fig. 1) indicate that the dissociation process does not have the usual characteristics of an indirect effect. Whether or not the protein is degraded before it dissociates is not known.

Although nucleic acid by itself is very sensitive to denaturation by γ-rays (1·6 eV./phosphorus atom causes 70 per cent denaturation[3]), there was no evidence for denaturation of the nucleic acid in the irradiated nucleoprotein even up to 40 eV./phosphorus atom. This agrees with the chemical evidence of Emmerson *et al.*[10] that up to dosages of $1{\cdot}3 \times 10^5$ rads (≃ 50 eV./phosphorus atom with their 0·02 per cent solutions) practically no radicals actually pene-

trate to the nucleic acid and attack either the bases or the phospho-ester bonds. It is also consistent with investigations on electron spin resonance made by Kirby-Smith[11]; these detect radical attack and suggest that ionizing radiation should at first damage the protein of a nucleoprotein complex. Thus it seems unlikely that γ-irradiation would readily cause denaturation of the nucleic acid in the nucleoprotein, for this is now known to be effected by the free radicals[12].

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[1] Cox, R. A., Overend, W. G., Peacocke, A. R., and Wilson, S., *Proc. Roy. Soc.*, B, **149**, 511 (1958).
[2] Peacocke, A. R., and Preston, B. N., *Proc. Roy Soc.*, B, **153**, 90 (1960).
[3] Peacocke, A. R., and Preston, B. N., *Proc. Roy. Soc.*, B, **153**, 103 (1960).
[4] Zubay, G., and Doty., P., *J. Mol. Biol.*, **1**, 1 (1959). Doty, P., and Zubay, G., *J. Amer. Chem. Soc.*, **78**, 6207 (1956).
[5] Bayley, P. M., Preston, B. N., and Peacocke, A. R. (unpublished work).
[6] Kaufmann, B. P., McDonald, M. R., and Bernstein, M. H., *Ann. N.Y. Acad. Sci.*, **59**, 553 (1955).
[7] Hems, G., *Nature*, **189**, 849 (1961).
[8] Kay, E. R. M., Simmons, N. S., and Dounce, A. L., *J. Amer. Chem. Soc.*, **74**, 1724 (1952).
[9] Wilkins, M. H. F., Zubay, G., and Wilson, H. R., *J. Mol. Biol.*, **1**, 179 (1959).
[10] Emmerson, P., Scholes, G., Thomson, D. H., Ward, J. F., and Weiss, J., *Nature*, **187**, 319 (1960).
[11] Kirby-Smith, J., *Radiation Res.*, **11**, 448 (1959).
[12] Lloyd, P., and Peacocke, A. R. (unpublished observations).

TRANSMISSION OF MOUSE LEUKÆMIA WITH PURIFIED NUCLEIC ACID PREPARATIONS

By Dr. ESTHER F. HAYS*

Laboratory of Nuclear Medicine and Radiation Biology, School of Medicine, University of California

LEUKÆMIA in the mouse has been demonstrated to be transmitted by hereditary factors. There exist certain inbred strains where the spontaneous incidence of this disease is high. Because of known genetic transmission of leukæmia I have attempted to transmit the disease with purified nucleic acid preparations from both normal and leukæmic tissues of mice of one of these inbred strains with a high spontaneous incidence of the disease.

Gross[1], in 1951, demonstrated that leukæmia of *AKR* mice could be transmitted to *C3H$_f$/Bi* mice if they were inoculated with cell-free filtrates of *AKR* leukæmic tissues when new-born. Law[2] was able to increase the incidence of spontaneous leukæmia in (*C3H$_f$* × *AKR*)F_1 hybrid mice from 27·7 per cent in control animals to 60·9 per cent in experimental animals by implanting in them fragments of normal thymic tissue from young *AKR* mice. Stewart[3] increased the incidence of leukæmia from 23 per cent to 60 per cent in this same hybrid cross by inoculating cell-free filtrates of *AKR* leukæmic tissues into new-born animals. Because of the work of these investigators indicating strain susceptibility of the (*C3H$_f$* × *AKR*)F_1 hybrid and *C3H$_f$/Bi* mice to transmission of *AKR* leukæmia, new-born hybrid mice and *C3H$_f$/Bi* mice were utilized as recipient animals for nucleic acids prepared from tissues of normal and leukæmic *AKR* mice.

Preliminary experiments[4] carried out in this Laboratory showed that when nucleic acids were injected subcutaneously into suckling mice less than 24 hr. of age of an F_1 hybrid of a *C3H$_f$* × *AKR* cross, there was an increased incidence of leukæmia in the injected animals when compared with the controls. Nucleic acids injected into new-born *C3H$_f$/Bi* mice did not result in leukæmia.

In the work to be reported here it has been possible to demonstrate, with larger numbers of animals than previously reported, that there is a significant increase in the incidence of leukæmia in (*C3H$_f$* × *AKR*) F_1 hybrid mice when injected with purified nucleic acid preparations from lymphoid tissues of *AKR* mice. These nucleic acids have not been associated with leukæmia development in *C3H$_f$/Bi* mice, but other tumours have been seen to develop in one small group of these mice injected with nucleic acid preparations into the thymus.

The *AKR/JAX* mice used as a source of spontaneous leukæmia were from an inbred colony that has been maintained in this Laboratory for four years. The original litter came from the Jackson Memorial Laboratory, Bar Harbor, Maine.

The *C3H$_f$/Bi* mice have been maintained as an inbred strain for five years, the original litter being a gift from Dr. L. Gross, Veterans Administration Hospital, Bronx, New York.

The F_1 hybrid mice were a cross between *AKR* males and *C3H* females of these two strains.

The animals were housed in wire-mesh cages containing stainless-steel-sponge basins with wood shavings for nests. They were supplied with Rockland rat diet and water *ad libitum* and the pregnant females were given lettuce and oatmeal once weekly. The new-born mice used for inoculation were 12–14 hr. of age in the subcutaneously injected group and 24–72 hr. of age in the intravenously injected group. The subcutaneous injections were made on the back with a 30-gauge needle and sealed with collodion in most cases. The intravenous injections were made with a 33-gauge needle into the orbital vein. The animals were observed until natural death. Autopsies were performed in all cases and appropriate organs taken for microscopic investigation when indicated.

The nucleic acids were prepared by the method of Simmons as previously described[4]; preparations used for intravenous injection substituted xylene sulphonate for sodium dodecyl sulphate as a detergent. The final nucleic acid yield from 5 gm. of tissue was dissolved in physiological saline and stored as a 1 per cent solution in 1 ml. aliquots at −10° until used. The preparations contained both deoxyribonucleic acid and ribonucleic acid. No attempt was made to separate the nucleic acids in these experiments. These preparations will be referred to as DNA–RNA.

* Present address: Department of Biophysics and Nuclear Medicine, University of California School of Medicine, 900 Veteran Avenue, Los Angeles 24, California.

The leukæmias in both experimental and control animals were grossly and microscopically similar to the disease which was seen to occur spontaneously in the *AKR* mice. The morphology of the tumour was similar to that described by Dunn[5] as a lymphocytic neoplasm or lymphocytic leukæmia.

Experiments with (C3H$_f$ × AKR)F$_1$ *Hybrid Mice.* The first group of animals was injected subcutaneously with DNA–RNA. (*C3H$_f$* × *AKR*)F_1 hybrid mice of 12–48 hr. of age were used. The results are summarized in Table 1. Forty-five animals were injected with 0·1 ml. of DNA–RNA. Seventeen (37·7 per cent) of these animals developed leukæmia. Nineteen had received nucleic acids prepared from pooled spleen and lymph nodes of non-leukæmic *AKR* mice with eight developing leukæmia. Twenty-six animals had received DNA–RNA from leukæmic *AKR* tissues (spleen, thymus and lymph node). Nine of these developed leukæmia. 175 animals were from non-injected litters of the same hybrid cross. These animals were raised concomitantly with the experimental animals and under the same conditions. Thirty-five animals in this group developed leukæmia, an incidence of 20 per cent. The mean age of development of leukæmia was approximately 12 months in both groups. The onset of the disease was 6–22 months in the injected group and 8–20 months in the controls. These results give a P value of less than 0·01 and confirm the results obtained in the preliminary experiments reported elsewhere[4].

Table 1. Results of Injection of Purified Nucleic Acid Preparations from *AKR* Mice into (*C3H$_f$* × *AKR*)F_1 Hybrid Mice at 12–72 Hr. of Age

Amount and route of injection	No. of animals	No. of leukæmias observed	Percentage
0·1 ml. DNA–RNA subcutaneously	45	17	37·7
No injection	175	35	20·0
0·05 ml. DNA–RNA intravenously	54	20	37·0
No injection	108	25	23·0

A second group of (*C3H$_f$* × *AKR*)F_1 hybrid animals was injected intravenously with 0·05 ml. of DNA–RNA from leukæmic *AKR* tissues. The injections were made in the orbital vein at 24–72 hr. of age. The controls again were alternate non-injected litters. The results of these experiments are included in Table 1. Fifty-four animals were injected with 0·05 ml. of DNA–RNA intravenously. Twenty of these animals (37 per cent) developed leukæmia at an average age of 10·5 months. 108 control mice were observed and 25 mice (23 per cent) developed leukæmia at an average age of 10·7 months. The P value for these results was less than 0·05.

Tumour cells from ten of the leukæmias developing in mice injected with DNA–RNA were implanted intraperitoneally into 2-month-old *C3H$_f$/Bi*, *AKR*, and hybrid mice. None of 20 *C3H* mice developed leukæmia. Four of 19 hybrid mice and four of 23 *AKR* mice developed leukæmia. In one case leukæmias occurred in *AKR* and hybrid mice receiving the same cell suspension. The leukæmias developed within 4 wk. of injection of cells and were assumed to be due to progressive growth of injected cells and not to have developed spontaneously.

Tumour transfers were carried out from cells of three leukæmias developing in control hybrid mice. No leukæmias developed in the 5 injected *C3H$_f$/Bi* and 4 hybrid mice, but 2 out of 4 injected *AKR* mice developed the disease within 4 wk. of transfer of cells. This would indicate that the leukæmias developing in the control and DNA–RNA injected mice had essentially the same transfer characteristics. However, the lack of consistent growth of these tumours in hybrid mice is difficult to explain.

Experiments with C3H$_f$/Bi *Mice.* A group of similar experiments was carried out utilizing the *C3H$_f$/Bi* mice. Ninety mice were injected intravenously with DNA–RNA at 12–72 hr. of age. Two leukæmias developed at 11 months.

Three litters were injected with 0·05 ml. of a DNA–RNA preparation to which had been added an equal volume of 50 per cent sucrose. These animals were injected into the thymus at 2–5 days of age. The majority of the animals died during the first 24 hr. after injection. However, five animals in this group survived. Three of these animals developed parotid gland carcinomas and one developed an adrenal gland neoplasm. The parotid tumours occurred at 7, 9 and 10 months of age and were local bilateral growths. The adrenal tumour was found at 10 months of age. It was metastasized to the liver and thoracic lymph nodes. The remaining mouse died of pneumonia at four months of age.

Four litters were injected with 0·05 ml. of cell-free 'Selas'-filtered extract of *AKR* leukæmic tissues into the thymus at 2–5 days of age. Eight of these animals survived. Their litter mates died during the first 24 hr. after injection. The cell-free extract was prepared as that described previously[6]. None of the eight animals that had received cell-free extract developed neoplasms or leukæmia.

One leukæmia was found in 114 non-injected control *C3H$_f$/Bi* mice raised at the same time and under the same conditions as the experimental animals.

Other work[4] had shown that the subcutaneous injection of DNA–RNA at 12–24 hr. of age in seventeen *C3H$_f$/Bi* animals did not result in leukæmia development. However, cell-free ('Selas'-filtered) extracts when injected into 36 mice of this strain resulted in the development of ten leukæmias. No leukæmias were seen in non-injected control animals.

A summary of the results of these experiments with *C3H$_f$/Bi* mice is given in Table 2. The tumours that developed in the DNA–RNA injected mice are grossly and histologically similar to those described by Stewart[7] as occurring in (*C3H$_f$* × *AKR*)F_1 hybrid mice after injection with cell-free extracts and later shown by Stewart and Eddy to be due to the polyoma virus[8]. Neoplasms of the parotid and adrenal gland have not been noted to occur spontaneously in the *C3H$_f$/Bi*, *AKR* or hybrid colonies.

These experiments demonstrate that the injection of nucleic acid preparations from normal and leukæmic *AKR* mice into new-born (*C3H$_f$* × *AKR*)F_1 hybrid mice is associated with a statistically significant increase in the incidence of leukæmia in the injected mice when compared with non-injected controls.

Table 2. Results of Injection of Purified Nucleic Acid Preparations compared with those of Cell-Free Extracts and Non-Injected Controls in *C3H$_f$/Bi* Mice

Material	Age injected	Route of injection	No. of animals	No. of leukæmias	Other tumours
Cell-free extract	12–24 hr.	Subcutaneous*	36	10	0
Cell-free extract	2–5 days	In thymus	8	0	0
DNA–RNA	12–24 hr.	Subcutaneous*	17	0	0
DNA–RNA	12–72 hr.	Intravenous	90	2	0
DNA–RNA	2–5 days	In thymus	5	0	4†
No injection			114	1	0

*These results are taken from previously published experiments (ref. 4).
†3 Parotid tumours, 1 adrenal tumour.

However, nucleic acids from leukæmic *AKR* mice prepared and injected in the same manner do not increase the incidence of leukæmia in *C3H$_f$/Bi* mice.

These two observations would seem to indicate that an inherited tendency to develop leukæmia in the hybrid mouse can be accentuated by the addition of nucleic acids from lymphoid tissues of a high-incidence leukæmia strain in the new-born period. This is possibly an observation of the effects of addition of more *AKR* genetic material with leukæmogenic properties to that of a mouse with an inherited leukæmic tendency from its *AKR* parent.

The *C3H$_f$/Bi* mouse is not susceptible to leukæmia induction with nucleic acids but is susceptible to induction of the disease with cell-free extracts of *AKR* tissues, presumably containing a virus[1]. This would suggest that viral nucleic acid is not involved in the observations made in the hybrid strain and that the situation is more analogous to that observed in bacterial transformation where the DNA of the donor combines with that of the recipient, resulting in the appearance of donor characteristics in the recipient bacterium. That is, the hybrid mice, after having received *AKR* nucleic acid, are more like their *AKR* parent, so far as tendency to develop leukæmia is concerned, than non-injected F_1 hybrids.

The observation of the induction of parotid and adrenal tumours in *C3H$_f$/Bi* mice injected into the thymus with nucleic acid from *AKR* leukæmic tissues would indicate that this nucleic acid preparation very likely contained DNA from polyoma virus. This preparation was made from a different *AKR* leukæmic mouse than that utilized for the subcutaneous and intravenous injections. An agent that induced parotid gland and other neoplasms in *C3H$_f$/Bi* mice was found initially in cell-free extracts of tissues of the leukæmic *AKR* mouse[9]. Latarjet[10] has carried out experiments using the detergent method of isolating nucleic acids from *AKR* leukæmic tissues. He also found no increased incidence of leukæmia in *C3H* mice injected subcutaneously with these nucleic acid preparations at birth, but 5 per cent of the injected animals after an average latency of 202 days developed multiple tumours which were interpreted by the author as being due to the inclusion of DNA from the polyoma virus in his preparations. The experiments of Di Mayorca *et al.*[11] and Smith *et al.*[12] have shown the polyoma virus to be a DNA virus.

Experiments are now in progress to investigate the effects of nucleases on the induction of leukæmia and other tumours with these nucleic acid preparations. A group of hybrid mice receiving nucleic acid preparations with litter-mate controls is under investigation.

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[1] Gross, L., *Proc. Soc. Exp. Biol. Med.*, **76**, 27 (1951).
[2] Law, L. W., *J. Nat. Cancer Inst.*, **12**, 789 (1952).
[3] Stewart, S. E., *J. Nat. Cancer Inst.*, **16**, 41 (1955).
[4] Hays, E. F., Simmons, N. S., and Beck, W. S., *Nature*, **180**, 1419 (1957).
[5] Dunn, T. B., *J. Nat. Cancer Inst.*, **14**, 1281 (1954).
[6] Hays, E. F., and Beck, W. S., *Cancer Research*, **18**, 6, 676 (1958).
[7] Stewart, S. E., *J. Nat. Cancer Inst.*, **15**, 1391 (1955).
[8] Stewart, S. E., Eddy, B. F., Gochenour, A. M., Borgese, N. G., and Grubbs, G. E., *Virology*, **3**, 380 (1957).
[9] Gross, L., *Proc. Soc. Exp. Biol. Med.*, **83**, 414 (1953).
[10] Latarjet, R., *Cancer Research*, **20**, 807 (1960).
[11] Di Mayorca, G. A., Eddy, B. E., Stewart, S. E., Hunter, W. S., Friend, C., and Bendich, A., *Proc. U.S. Nat. Acad. Sci.*, **45**, 1805 (1959).
[12] Smith, J. D., Freeman, G., Vogt, M., and Dulbecco, R., *Virology*, **12**, 185 (1960).

ENHANCED EFFECT OF TOTAL-BODY X-IRRADIATION IN MICE UNDER MILD HYPOTHERMIA

By Dr. H. J. G. BLOOM

Radiotherapy Department, Royal Marsden Hospital, and Institute of Cancer Research, Royal Cancer Hospital, London, S.W.3

AND

K. B. DAWSON

Radiobiology Research Unit, Institute of Cancer Research, Royal Cancer Hospital, London, S.W.3

IT is well established that the sensitivity of a variety of biological species to damage by ionizing radiation is dependent on the presence or absence of oxygen in the environment at the time of irradiation. An increase in tissue oxygen can be brought about through breathing oxygen under raised pressure or by lowering its consumption by means of hypothermia.

The use of hypothermia in man to raise the cerebral oxygen concentration and thereby perhaps increase the radiosensitivity of intracranial tumours is being investigated at the Royal Marsden Hospital, London. This has been undertaken to try to improve the extremely poor results obtained by conventional methods of treatment in patients suffering from malignant grades III and IV cerebral astrocytomata (glioblastoma multiforme). Under generalized mild hypothermia (30–32°) moderate doses of whole brain X-irradiation (300–450 r. given in daily fractions of 50–150 r.) appear to produce an exaggerated cerebral reaction[1]. Many problems were encountered in this treatment and, while the clinical work was in progress, the present investigation was undertaken to investigate the influence of hypothermia on the response of mice to whole-body irradiation.

Eight-week-old albino male mice of Theiler's original strain were anæsthetized with intraperitoneal sodium pentobarbitone (approximately 50 mgm./kgm. body-weight) and then suspended by a plastic collar in a thermostatically controlled water-bath, the entire body with the exception of the head being immersed. The bath was kept at various temperatures between 29° and 37° (Table 1) and the colonic temperature of each mouse was continuously recorded by a mercury thermometer *in situ*. The temperatures of the anæsthetized mice fell to approximately 29° (room temperature, 20°) and remained in this region until

they were placed in the water-bath; the colonic temperatures reached that of the bath within 10 min. of immersion.

The mice were irradiated five at a time in the water-bath and allowed to remain immersed for 20 min. before and after the irradiation. The X-ray factors were: 140 kV.; 5 m.amp.; focal skin distance 38 cm.; no added filtration; half-value layer 2 mm. aluminium; dose-rate 25 r./min. After removal from the bath the mice were placed in a box at 37° to recover and dry out.

The effects of total-body irradiation with a dose of 550 r. was studied, but the head, not being shielded by the water, received 12 per cent more irradiation. For each of the temperatures used, 10 control mice were treated under identical conditions, but shielded in each case from irradiation by a 3-mm. thick lead screen.

In these experiments the criterion of an irradiation effect was death of the animal within 30 days. The majority of fatalities occurred between the tenth and twentieth day following irradiation. There did not appear to be any difference in the mode of death at the different temperatures, nor with the number of animals involved could a significant relationship be established between time of death and temperature.

The results (Table 1) show an increased mortality among the animals treated under mild hypothermic conditions (31–35°). At a temperature of 29° a protective effect was noted. No deaths occurred in any of the control animal groups.

Table 1. Effect on Mouse Survival of Combined Hypothermia and 550 r. Total-Body X-irradiation

Temperature	No. of mice	Survivals	Died (per cent)
29°	19	19	0
31°	19	7	63
33°	18	11	39
35°	18	13	28
37°	21	18	14

Ten control animals for each temperature, shielded from irradiation: no deaths.

Hypothermia, by lowering the metabolic rate and by increasing the amount of oxygen dissolved in the plasma, may lead to an increase in the oxygen environment of tissues and thereby perhaps an increase in radiosensitivity. Working against these tendencies to an increased oxygen tension in hypothermia, however, may be: (1) a reduction of blood-flow due to the decreased cardiac output, the increased peripheral resistance and the increased viscosity of the blood; (2) a shift of the oxygen dissociation curve to the left, which will interfere with the liberation of oxygen from hæmoglobin; (3) a fall in the rate and depth of respiration. On the other hand, normal well-oxygenated tissues are considered to be practically at their maximum radiosensitivity. In such circumstances no appreciable increase in response to ionizing radiation is expected to follow a further rise of oxygen tension, as opposed to relatively anoxic foci in tumours which have outgrown their blood supply[2]. The oxygen tension in normal bone marrow, however, appears to be low compared with that of muscle and subcutaneous tissue[3]. If this is true, hypothermia may increase the oxygen environment and therefore the radiosensitivity of normal marrow. The chief cause of death in mice following 550 r. whole-body irradiation is marrow failure. Alternatively, the lowered temperature may increase radiosensitivity by interfering with the efficiency of detoxifying and repair mechanisms or the transport of tissue breakdown products. A third possibility is that hypothermia, like mild whole-body irradiation, may increase the response to local irradiation[4] through the production of certain plasma factors[5]. A recent report[6] suggests that a greater difference in litter size, in viability at birth, in developmental curves and in behaviour changes occur in chicks following whole-body pre-natal irradiation of the mother compared with localized direct irradiation of the gravid uterus.

The influence of temperature on the degree and the timing of response to γ- and X-ray irradiation of various biological systems have been investigated by many workers with somewhat conflicting results. In general, profound hypothermia (15° C. or less) reduces the damage resulting from irradiation, and such observations have recently been reviewed by Weiss[7]. Heat, on the other hand, appears to have an additive effect on irradiation[8,9].

Although most investigators report that hypothermia reduces radiosensitivity, an increased effect at reduced temperatures has been reported in the broad bean root[10], in *Tradescantia* microspores[11], in mouse skin[12] and in mouse carcinoma tissue[13]. Changes of temperatures at the time of irradiation do not appear to influence the response of isolated mammalian cells *in vitro*[14–16], but may bring about pronounced effects in more complex tissue systems during *in vivo* studies[7,17–20].

The results of the present investigation appear to support a protective effect for hypothermia at 29° against ionizing radiation, but reveal an enhanced effect of irradiation with a lesser degree of hypothermia, the peak being at 31–32°. The difference in survival-rate following whole-body irradiation at 31° and 29°, a change of only 2 degrees, is striking. The results in mice reported here and the severe cerebral reactions observed in patients treated by whole-brain irradiation[1] suggest that the increased response to irradiation during mild hypothermia is more likely to be due to systemic humoral factors rather than a local oxygen effect (for example, stress endocrine changes; tissue breakdown products).

These experiments, including an investigation into the possible mechanisms involved, will be reported in greater detail elsewhere.

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[1] Bloch, M., Bloom, H. J. G., Penman, J., and Walsh, L. S., *Lancet*, ii, 907 (1961).
[2] Gray, L. H., *Lectures on the Scientific Basis of Medicine*, **7**, 314 (University of London, 1957).
[3] Cater, D. B., and Silver, I. A., *Acta radiol.*, **53**, 233 (1960).
[4] Hollcroft, J. W., Lorenz, E., and Hunstiger, H., *J. Nat. Cancer Inst.*, **11**, 1 (1950).
[5] Sikov, M. R., and Lofstrom, J. E., *Amer. J. Roent.*, **84**, 705 (1960).
[6] Meier, G. W., *Symposium on Effects of Ionizing Radiation on the Nervous System*, International Atomic Energy Agency, Vienna, May 5–9 (1961).
[7] Weiss, L., *Brit. Med. Bull.*, **17**, 70 (1960).
[8] Selawry, O. S., Carlson, J. C., and Moore, G. E., *Amer. J. Roent.*, **80**, 833 (1958).
[9] Modlin, R. K., and Morris, J. M., *Cancer*, **14**, 117 (1961).
[10] Mottram, J. C., *Brit. J. Radiol.*, **8**, 32 (1935).
[11] Sax, K., and Enzmann, E. V., *Proc. U.S. Nat. Acad. Sci.*, **25**, 397 (1939).
[12] Mottram, J. C., *Brit. J. Radiol.*, **29**, 174 (1924).
[13] Crabtree, H. G., and Cramer, W., Eleventh Scientific Rep. Imp. Cancer Res. Fnd., 89, 103 (1934).
[14] Weiss, L., *Intern. J. Rad. Biol.*, **2**, 20 (1960).
[15] Alexander, P., Thirty-eighth Ann. Rep., British Empire Cancer Campaign, 79 (1960).
[16] Shapiro, I. M., *Intern. J. Rad. Biol.*, **3**, 293 (1961).
[17] Hajduković, S., Hervé, A., and Vidović, V., *Experientia*, **10**, 343 (1954).
[18] Kuskin, S. M., Wang, S. C., and Rugh, R., *Amer. J. Physiol.*, **196**, 1211 (1959).
[19] Weiss, L., *Brit. J. Radiol.*, **33**, 32 (1960).
[20] Weiss, L., *Intern. J. Rad. Biol.*, **3**, 149 (1961).

SIMPLIFIED TECHNIQUE FOR ISOLATION OF SUB-CELLULAR PARTICLES

By Dr. DAVID W. E. SMITH*

Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda 14, Maryland

THE heterogeneity of particles isolated in the classical sub-cellular fractions[1-3] makes it desirable that tissue homogenates be divided into a greater number of fractions each of which can be tested for enzymic activity and chemical composition. Density gradient centrifugation has been widely used for this, but it has certain disadvantages. These are: difficulty in constructing reproducible gradients, especially of the continuous variety, difficulty in estimating the sizes of the particles collected[4], and difficulty in collecting the particles from the various levels in the gradient after centrifugation. Moreover, in order to construct gradients of sufficient density to separate particles, many workers have used extremely hypertonic solutions which have probably had deleterious effects on the morphology and activity of the particles collected.

My technique utilizes layering of aliquots of a homogenate in 0·88 *M* sucrose over relatively large amounts of clear 0·92 *M* sucrose solution, and centrifugation of a series of these preparations at successively greater forces. Each increased force sediments all the particles sedimented by previous forces, together with some smaller particles not sedimented by the lower forces. The value of using 'two phase' preparations has been demonstrated[5]. Only particles which sediment completely are collected in the precipitate. Smaller particles are decanted with the supernatant, and since all the particles start sedimenting at essentially the same level at the top of the tube, there is negligible contamination of desired particles in the precipitate with smaller particles. The clear phase, moreover, washes the particles as they sediment and frees them of contaminants.

DeDuve *et al.*[6] have shown that the integral force applied to a preparation can be approximated by multiplying the force used by the time during which it was applied. The product is expressed in gravity-minute (*g*-min.) units. I have found it desirable to use the swinging bucket rotor (*SW* 39) in the 'Spinco' model *L* centrifuge because sedimenting particles in angle rotors move first to the centrifugal side of the tube and then slide down the tube to the bottom. In the horizontal rotor the distance that a particle must move from the thin layer of homogenate to the bottom of the tube can be measured accurately, and at the end of centrifugation there are fewer particles adhering to the sides of the tube.

No special precautions were taken to prevent vibration during acceleration and deceleration of the rotor. Such vibration would appear to be less critical in this procedure, with a packed sediment well separated from suspended particles, than in density gradient methods, where zones of suspended particles must be stabilized. The acceleration and deceleration times were short relative to centrifugation time at top speed, and only the latter was used in the calculation. To test the efficacy of our technique, a survey of uricase and cytochrome *c* oxidase in the particles of rat liver was done. Particles were sedimented by layering 0·5 ml. of 20 per cent rat liver homogenate in 0·88 *M* sucrose over 3·0 ml. of 0·92 *M* sucrose and centrifuging a series of these preparations for varying periods of time at different speeds. Aliquots of the supernatants were used for enzymic assays. Cytochrome *c* oxidase was assayed by the technique described by Smith[7]. Uricase was assayed by the technique of Hogeboom and Schneider[8] with modifications as suggested by Kalckar[9]. The results are shown in Fig. 1. The activity of the enzyme in each supernatant is plotted against the integral centrifugal force used to prepare it. The centrifugal forces shown are those applied at the bottom of the tube. The curves thus show the disappearance of these enzymes from the homogenate and indicate the forces required to precipitate the particles containing the enzymes.

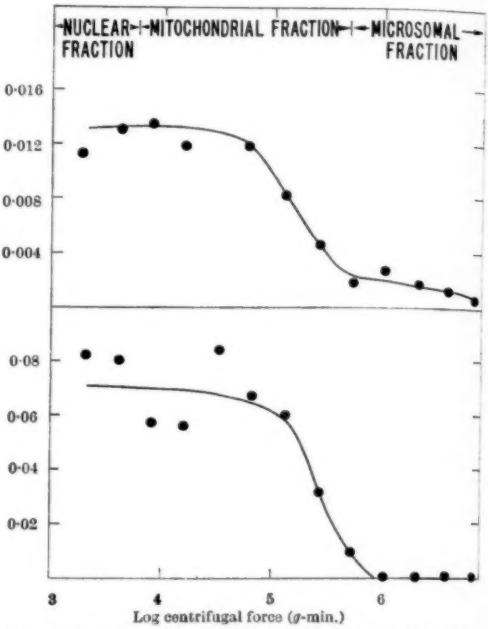

Fig. 1. Distribution of uricase and cytochrome *c* oxidase in sub-cellular particles of rat livers

Ordinates, cytochrome *c* oxidase activity (top) is given as the constant for the first order reaction observed colorimetrically in sec.$^{-1}$ units; uricase activity (bottom) as micromoles of uric acid destroyed by each fraction in 30 min.

Comparison of the forces needed to precipitate these enzymes with those used to prepare classical sub-cellular fractions[10] (indicated at the top of Fig. 1) shows that both of the enzymes are 'mitochondrial'. The range of forces precipitating particles containing either of these enzymes is much narrower, however, than the range used to prepare the whole mitochondrial fraction. From a knowledge of these forces, a fractionation method can be devised by which particles containing either of these enzymes can be isolated in the absence of other particles usually isolated with the 'mitochondrial fraction'.

*Present address: Department of Pathology, Yale University School of Medicine, New Haven, Connecticut.

Calculation of the diameters of the particles (assuming that they are spherical) can be made using the following expression, which may be derived from the equations given by DeDuve and Berthet[6]:

$$d^2 = \frac{63\ \eta\ (\log_{10} r_2 - \log_{10} r_1)}{(\sigma - \rho)\ (\text{r.p.m.})^2\ T}$$

where d = particle diameter (cm.), η = viscosity of the suspending medium (poises), T = time for sedimentation (min.), σ = particle density (gm./c.c.), ρ = density of the suspending medium (gm./c.c.), r_1 = the distance from the axis of rotation to the surface of the suspension in the tube (cm.), r_2 = the distance from the axis of rotation to the bottom of the centrifuge tube (cm.), and r.p.m. = the speed of centrifugation (revolutions per minute).

The value for the density of these particles was that determined in sucrose and ordinary water by Beaufay *et al.*[11]. The approximate average density of 1·22 was used for both cytochrome *c* oxidase and uricase, although the uricase particles appear to be less homogeneous. Since this value was determined by isopycnic centrifugation, the density of any particle undergoing osmotic shrinking will be increased relative to that in isotonic solutions or in 0·92 *M* sucrose used in the present experiments. Its diameter, calculated from the above equation, will be underestimated. Density values obtained in sucrose–deuterium oxide gradients[11] were not used because of the increased density of uricase apparently due to bound deuterium oxide. The values for the density and viscosity of 0·92 *M* sucrose were obtained from DeDuve *et al.*[12].

The diameters calculated from the data in Fig. 1 showed a range of 0·52–1·58μ for cytochrome *c* oxidase-containing particles, and 0·26–0·75μ for those containing uricase. These may be compared with the values reported by Beaufay *et al.*[11], where calculation was made by reference to 'standard conditions' of 0·25 *M* sucrose and 0° C. They found the diameter of cytochrome *c* oxidase-containing particles to be about 0·5–1·2μ and of uricase-containing particles, 0·2–0·7μ. Other workers have also found the 'mitochondrial' oxidative enzymes to be associated with larger particles than the 'mitochondrial' (lysosomal) lytic enzymes[10,13].

[1] Chantrenne, H., *Biochim. Biophys. Acta*, **1**, 437 (1947).
[2] DeDuve, C., Pressman, B. C., Gianetto, R., Wattiaux, R., and Appelmans, F., *Biochem. J.*, **60**, 604 (1955).
[3] Kuff, E. L., and Schneider, W. C., *J. Biol. Chem.*, **206**, 677 (1954).
[4] Thomson, J. F., and Klipfel, F. J., *Exp. Cell Res.*, **14**, 612 (1958).
[5] Hogeboom, G. H., Schneider, W. C., and Striebich, M. H., *Cancer Res.*, **13**, 617 (1953).
[6] DeDuve, C., and Berthet, J., *Nature*, **172**, 1142 (1953).
[7] Smith, L., in *Methods in Enzymology*, 11 (Acad. Press, New York, 1955).
[8] Hogeboom, G. H., and Schneider, W. C., *J. Biol. Chem.*, **195**, 161 (1952).
[9] Kalckar, H. M., *J. Biol. Chem.*, **167**, 461 (1947).
[10] Hogeboom, G. H., Schneider, W. C., and Pallade, G. E., *J. Biol. Chem.*, **172**, 622 (1948).
[11] Beaufay, H., Bendall, D. S., Baudhuin, P., Wattiaux, R., and DeDuve, C., *Biochem. J.*, **73**, 628 (1959).
[12] DeDuve, C., Berthet, J., and Beaufay, H., *Prog. Biophys.*, **9**, 325 (1959).
[13] Kuff, E. L., Hogeboom, G. H., and Dalton, A. J., *J. Biophys. Biochem. Cytol.*, **2**, 33 (1956).

ALTERATIONS IN CONNECTIVE TISSUE AND INTESTINE PRODUCED BY HYPERVITAMINOSIS A IN *XENOPUS LAEVIS*

By GERALD WEISSMANN

Strangeways Research Laboratory, Cambridge

RECENT experiments have indicated that vitamin A may liberate from intracellular lysosomes a cathepsin-like protease active on such extracellular materials as cartilage matrix[1]. Thus the depletion of cartilage matrix observed in rabbits made acutely hypervitaminotic A resembled that produced by an exogenously administered protease, papain[2]. Considerable catheptic activity has been demonstrated in the tails of amphibian larvæ; this is greatest at the time of tail resorption during metamorphosis[3]. If the hypothesis of vitamin A action on lysosomes is correct, resorption of the tails of amphibian larvæ might accompany hypervitaminosis A, since the vitamin would cause release of cathepsins from the lysosomes, which Novikoff has shown are abundant in amphibian tails[4].

Larvæ of *Xenopus laevis* were therefore exposed to high doses of vitamin A, and the effect of this treatment on gross and microscopical structure was observed. Although the predicted effects on connective tissue were seen, and will be briefly noted, this article is chiefly concerned with an entirely unexpected effect of this régime on the intestines of the larvæ.

Larvæ of *Xenopus laevis*, obtained through the generosity of Dr. R. T. Sims, Department of Anatomy, University of Cambridge, were grown under conditions suggested by Nieuwkoop and Faber[5], at room temperature and fed with nettle powder thrice weekly. Twenty-eight animals were given 2 mgm. of synthetic vitamin A alcohol ground with their food twice a week; the tank contained 800 ml. of tap water for each group of 14 larvæ. An equal number served as controls.

Treatment was continued for three months, beginning with larval stages 49–50. By the end of the experiments, the treated animals had progressed to stage 59–61, while some of the controls had undergone metamorphosis. After three weeks, six of the experimental animals were transferred to fresh water, and given the normal diet without added vitamin A. At varying intervals, larvæ were killed with 'Nembutal', fixed *in toto* in acetic alcohol, embedded, and sections were stained with hæmatoxylin and eosin and with toluidine blue.

Grossly, the first change noted was a drooping of the tentacles of the larvæ exposed to the vitamin. This occurred on the fourteenth or fifteenth day of treatment, and resembled in many ways the drooping of the ears of rabbits made hypervitaminotic A, or of those given papain intravenously[2]. After 18–20 days, however, another change became evident. The stools of the animals, which had been well-formed, dark, and solid, assumed a mucinous, thready appearance and floated in strands at the surface of the tank. This change persisted to the end of the experiment, and simple addition of vitamin A to a suspension of normal stools did not produce a similar

Fig. 1. Photomicrographs of fore-gut of *Xenopus laevis* larvæ (stage 53–54) stained with toluidine blue. (*a*) is from an animal exposed to high doses of vitamin A alcohol for 42 days. There is a marked overgrowth of metachromatically staining goblet cells, and the epithelium is more columnar than in the control (*b*) (× 216)

change. That this represented diarrhœa was readily observed by isolating individual normal and hypervitaminotic larvæ and noting the increased number of stools eliminated by the latter. By the twenty-first day of the experiment, most of the hypervitaminotic larvæ had developed pronounced kyphoscoliosis and also showed definite resorption of the tail, with a loss of the fine flagellar structure of that appendage; several actually developed fractures of the tails, along with shrivelling and resorption. Associated with the gross skeletal deformity was a change in the normal apposition of the upper and lower jaws, the experimental group having developed a prognathic appearance.

Histologically, the most remarkable change was in the gut of the hypervitaminotic animals. Sections of gut taken before the appearance of mucinous stools were unremarkable, but those taken two or three weeks later showed a great increase in the number of metachromatically staining goblet cells (Fig. 1*a*). These, situated mainly in the proximal gut and in the lower colon, were increased not only in absolute number (per high-power field in sections cut at 7μ) but also in ratio to the normal cells of the epithelium. These metachromatic cells were secreting discrete strands of metachromatic material into the lumen. The fæcal material in the gut of treated animals was diffuse, mucinous, and mixed with metachromatic strands, thus contrasting with similar debris in the lumen of controls where it was compact and entirely orthochromatic.

The whole character of the hypervitaminotic gut was altered. At comparable larval stages, the intestines of the treated animals had better developed villi, abnormally deep columnar epithelium (Fig. 1*a*, *b*) and at stages before metamorphosis (stages 49–60) the gut closely resembled the organ in control animals after metamorphosis, except for the remarkable overgrowth of goblet cells. In other structures, especially in the resorbing tail and the collapsed tentacles, there was a decrease of the metachromatically staining material of the ground substance; in these chronically hypervitaminotic animals the metachromasy of cartilage was almost unaffected, unlike that of acutely hypervitaminotic rabbits[3]. Those larvæ which were placed in the recovery group lost all the gross morphological stigmata of hypervitaminosis A within 21 days, and their gastrointestinal tracts were indistinguishable histologically from those of the controls, despite the continuance of mucinous diarrhœa for 8–10 days after transfer to a medium without vitamin A.

The above findings are in accord with two recently postulated effects of vitamin A. Moretti and Wolf[6] have found that mucopolysaccharide-bound hexosamine is decreased in the colon of hypovitaminotic A rats, and have suggested that vitamin A is necessary for mucopolysaccharide synthesis. It has been postulated that vitamin A causes the release of a proteolytic enzyme from cells or their organelles[2–7] which afterwards acts on the protein–polysaccharide complexes of connective tissue. In amphibian tails and tentacles, the means for their own resorption during metamorphosis must be 'built in' as an active lysosome–cathepsin system, and the observation that these structures are affected by vitamin A, would support the lysosomal theory of vitamin A action[1]. If the degradation of mucoprotein complexes in the epithelium of gut or in the ground substance of connective tissue by cell-released proteases were to act as a stimulus for the resynthesis of protein-polysaccharide complexes by competent cells, then the lysosomal action would explain the intestinal as well as the cartilagenous changes.

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[1] Dingle, J. T., and Lucy, J. A., *Biochem. J.*, **78**, 11P (1961).
[2] Thomas, L., McCluskey, R. T., Potter, J. L., and Weissmann, G., *J. Exp. Med.*, **112**, 705 (1960).
[3] Weber, R., *Rev. Suisse Zool.*, **64**, 326 (1957).
[4] Novikoff, A., in *Developing Cell Systems and Their Control*, 193 (Ronald Press Co., New York, 1960).
[5] Nieuwkoop, P. D., and Faber, J., in *Normal Tables of Xenopus Lævis* (North Holland Publ. Co., Amsterdam, 1956).
[6] Moretti, A., and Wolf, G., *Biochem. Biophys. Acta*, **46**, 392 (1961).
[7] Fell, H. B., and Thomas, L., *J. Exp. Med.*, **111**, 719 (1960).

SEPARATION OF BACTERIA BY COUNTER-CURRENT DISTRIBUTION

By G. DAVID BAIRD, PER-ÅKE ALBERTSSON and BENGT v. HOFSTEN
Institute of Biochemistry, University of Uppsala

THE method of counter-current distribution has been successfully applied to the separation of important biochemical substances[1]. Even very large particles, such as proteins of high molecular weight and viruses, may be studied by this method, if aqueous-phase systems containing two different water-soluble polymers are used[2–4]. In this case, the use of organic solvents, which may cause denaturation, is avoided.

In the present article we report some results of work on the distribution of whole cells, in this case bacteria, in these aqueous polymer phase systems. It has been found that the nature of the distribution in a given phase system of this type is frequently different for different bacteria. On the basis of this observation, it has been found possible to separate even quite closely related bacterial types by means of counter-current distribution. The

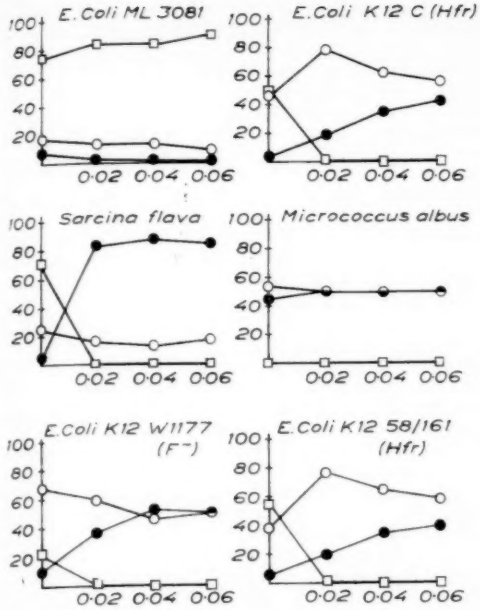


Fig. 1. Distribution patterns of different types of bacterium in phase system (D_5^{48}–PEG_4^{6000}) in the absence and presence of sodium chloride. Abscissa, concentration of sodium chloride (*M*); ordinate, quantity of cells present in each phase and at the interface (per cent of total added). Experiments carried out at 20° C. Bacteria cultured overnight on Penassay broth at 37° C., collected in growing state and afterwards washed twice in 0·01 *M* potassium phosphate buffer, *p*H 6·9, at 5° C. In general, the distribution of each bacterial type examined by mixing 1 ml. of washed cell suspension of *E*(1 cm., 400 mμ) = 8·0 with 9 gm. of aqueous polymer solution to produce about 10 gm. of cell/phase system mixture, in which the polymers were at their correct concentration. The bottom phase of this mixture had a volume of 4·6 ml. and the top phase a volume of 5·2 ml.
●, Bottom phase; ○, interface; □, top phase

results obtained may be of interest not only to microbiologists but also to other cell research workers, since in principle it should also be possible to apply the method to the separation of other types of whole cells.

Phase systems consisting of aqueous solutions of dextran and polyethylene glycol have been used for examining the distribution of bacteria. Systems containing these polymers have previously been employed mainly for studying the distribution of proteins[2,3]. The particular phase system that was found most appropriate for the studies on bacteria was that containing 5 per cent w/w dextran ('Dextran 500', limiting viscosity number 48 ml./gm., obtained from A. B. Pharmacia, Uppsala, Sweden) and 4 per cent w/w polyethylene glycol ('Carbowax 6000', average molecular weight 6,000–7,500, obtained from Carbide and Carbon Chemicals Co., New York). This phase system will be designated (D_5^{48} – PEG_4^{6000}) in this article. The aqueous solution of these polymers was lightly buffered with 0·01 *M* potassium phosphate buffer, *p*H 6·9. The distribution of proteins in the dextran/polyethylene glycol systems was found to be markedly influenced by the presence of electrolytes[2,3]. It seemed therefore of interest also to examine the effect of electrolytes on the distribution of bacteria. Accordingly, the effect of increasing concentrations of sodium chloride on the distribution of each bacterial type in (D_5^{48} – PEG_4^{6000}) was also studied.

The behaviour of a number of different types of bacteria under these conditions is illustrated in Fig. 1. In the case of *Escherichia coli ML* 3081, it can be seen that in the absence of sodium chloride the majority of the cells are suspended in the top phase, and that increasing concentrations of salt scarcely affect this distribution pattern at all. It appears that other *E. coli ML* strains also behave in a similar fashion. In the case of the *E. coli K* 12 *C* (*Hfr*) strain, too, a large portion of the cells are present in the upper phase in the absence of salt. Increase of salt concentration to 0·02 *M*, however, causes these cells to be transferred to the interface and the bottom phase. It seems to be a general rule that the distribution of bacterial cells in this phase system is more sensitive to increasing concentrations of sodium chloride in the region 0·00–0·02 *M* than at higher salt concentrations. Thus, the distribution of *Sarcina flava* is also altered markedly by increasing the concentration of sodium chloride to 0·02 *M*. In the absence of salt, the majority of the cells of this bacterium are present in the top phase, but are transferred to the bottom phase when 0·02 *M* sodium chloride is added. Increase of the salt concentration to 0·06 *M* does not appear to affect the distribution any further. *Micrococcus albus* is another example of a bacterium the distribution of which is scarcely affected by increase of concentration of sodium chloride from 0·00 to 0·06 *M*. In this case, about half the cells are present at the interface and about half in the bottom phase at all the salt concentrations used.

It is thus clear that different types of bacteria are distributed in quite different fashions in this phase system. However, on occasions different bacteria can also behave in the same manner. For example, the *E. coli K* 12 strains, 58/161 (*Hfr*) and C 600 (*F*⁻), and also *Bacillus megaterium* are distributed in this system in a very similar way to *E. coli K* 12 *C* (*Hfr*). (*F*⁻ indicates standard female mating type; *Hfr* indicates male giving high frequency of recombination.)

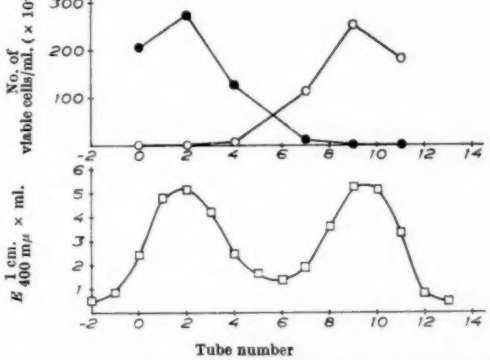


Fig. 2. Counter-current distribution of a mixture of *E. coli ML* 3081 and *E. coli K* 12 *C* (*Hfr*) in phase system (D_5^{48}–PEG_4^{6000}), containing 0·06 *M* sodium chloride. Cells cultured and washed as for Fig. 1. 1 ml. samples of *ML* 3081 and *C*(*Hfr*), each of *E*(1 cm., 400 mμ) = 26·0, added to tube No. 0. Twelve transfers carried out. Lower graph, variation in *E*(1 cm., 400 mμ) × total volume of cell/phase system mixture per tube (7·5 ml.) with tube number. Upper graph, variation in viable count on *EMB* agar plates with tube number. Volume of bottom phase 2·5 ml. and of top phase 5·0 ml., of which 0·5 ml. kept stationary. Total volume per tube therefore 7·5 ml. and stationary volume 3 ml. A hand-operated train of 20 glass tubes of the type described by Raymond (ref. 6) (obtained from E-C Apparatus Co., Swarthmore, Penn., U.S.A.) was used in this experiment, which was carried out at room temperature (20–23° C.)
●, lactose +ve colonies; ○, lactose −ve colonies

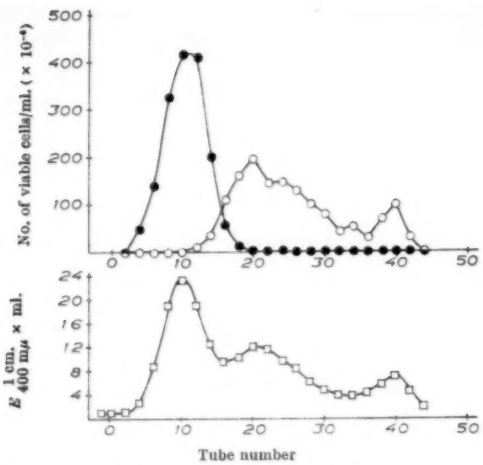


Fig. 3. Counter-current distribution of a mixture of the *E. coli* *K* 12 strains *W* 1177 (F^-) and 58/161 (*Hfr*) in phase system ($D_5^{4.8} - PEG_4^{6000}$) in the absence of sodium chloride. Cells cultured and washed as for Fig. 1. 5 ml. samples of *W* 1177 and 58/161, each of *E*(1 cm., 400 mμ) = 40·0, added to tube No. 0. Forty-five transfers carried out. Lower graph, variation in *E*(1 cm., 400 mμ) × total volume of cell/phase system mixture per tube (20·7 ml.) with tube number. Upper graph, variation in viable count on *EMB* agar plates with tube number. Volume of bottom phase 9·5 ml. and of top phase 11·2 ml., of which 0·5 ml. kept stationary. Total volume per tube therefore 20·7 ml. and stationary volume 10 ml. An automatically operated train of glass tubes of the type due to Craig (ref. 1) was used in this experiment, which was carried out at room temperature (20–23° C.)
●, lactose −ve colonies; ○, lactose +ve colonies

It can then be seen that it is possible to obtain a partial fractionation of cells from a mixture of bacteria which have different distribution patterns in the phase system merely by a one-step procedure. To obtain a more complete purification of the cells of one particular type of bacterium from such a mixture, this mixture can be subjected to counter-current distribution. The straightforward counter-current procedure, where the top phase is transferred and the bottom phase kept stationary, cannot, however, be used for bacterial cells, as they, in common with other whole cells, are usually to some extent adsorbed to the interface. Instead, the whole bottom phase, the interface and a small layer of the upper phase above it can be kept stationary, while the rest of the upper phase is transferred[2]. Alternatively, a small layer of the bottom phase, the interface and the whole of the top phase may be transferred, while the rest of the bottom phase is kept stationary. In either of these cases, distribution of the particles occurs effectively between the interface and that phase which is moving relative to it.

Several mixtures containing two different types of bacteria have been fractionated by this method of counter-current distribution, the results of two such experiments being illustrated in Figs. 2 and 3. In both these experiments, the two bacterial types were cultured and washed separately and then mixed, together with phase system, in the first tube of the distribution chain. The choice of pairs of bacteria for these two experiments was determined not only by differences in behaviour in phase system but also by the necessity of distinguishing between the members of each pair in a convenient fashion. This latter requirement was met by selecting pairs, one member of which could ferment lactose, and was therefore lactose-positive, while the other could not ferment this substance, and was therefore lactose-negative. It was then possible to distinguish between the two by means of the well-known procedure in which bacteria are plated out on agar containing both lactose and indicator (*EMB* agar). As the result of acid production during lactose fermentation, lactose-positive colonies appear dark red when grown on this agar, whereas the lactose-negative remain light pink.

From Fig. 1, it is clear that in phase system ($D_5^{4.8} - PEG_4^{6000}$), the majority of *E. coli* *ML* 3081 cells (lactose-negative) are found in the top phase in the presence of 0·06 *M* sodium chloride, while under these conditions *E. coli* *K* 12 *C* (*Hfr*) (lactose-positive) is found either at the interface or in the bottom phase. It should therefore be possible largely to separate a mixture of these two in the presence of this concentration of salt in one step, assuming that the distribution of each is unaffected by the presence of the other. Alternatively, complete separation should be obtained by counter-current distribution after only a small number of transfers. To demonstrate this, the experiment illustrated in Fig. 2 was performed. Here, the interface was kept stationary. At the conclusion of the experiment it was possible, as described above, to determine the nature and proportion of the bacteria in each tube of the train by plating out on *EMB* agar. From the plating results, it is clear that tubes 0–4 and 7–11 of the extraction train contained virtually uncontaminated samples of *K* 12 *C* (*Hfr*) and *ML* 3081 respectively.

It is also possible to fractionate mixtures of bacterial cells which have distributions which are much more similar to each other than those of *ML* 3081 and *K* 12 *C* (*Hfr*). Thus, mixtures of the *E. coli* *K* 12 strains *W* 1177 (F^-) (lactose-negative) and 58/161 (*Hfr*) (lactose-positive), the distributions of which are shown in Fig. 1, can also be successfully separated by the counter-current procedure. From Fig. 1 it can be seen that a greater portion of the male cells than of the female cells partition in favour of the top phase of the ($D_5^{4.8} - PEG_4^{6000}$) system when no salt is present. This implies that it is possible to separate the two strains using a stationary interface, if a sufficient number of transfers is carried out. Fig. 3 represents the results of an experiment carried out in this manner. From the viable counts on *EMB* agar it is apparent that the two cell types were largely separated in this experiment. One may assume that, with a larger number of transfers, complete separation could have been achieved. The reason for the asymmetric distribution of the *Hfr* cells is under investigation.

These results indicate, then, that different types of bacteria frequently have different patterns of distribution in the dextran/polyethylene glycol system. It would seem that these distribution patterns, each of which can be expressed as a phase-interface distribution ratio, G_i, can be maintained through a number of transfers in the counter-current apparatus, implying in particular that adsorption of cells to the interface is reversible[2]. Furthermore, the distribution of one type of bacterium seems not to be noticeably affected by the presence of another with a different sort of distribution. It can be noted here that comparison of viable counts of untreated bacteria and of bacteria which had been subjected to counter-current distribution indicated that treatment with phase system did not apparently have any deleterious effect on the viability of the bacterial cells.

It is of interest that even very similar strains, such as the *E. coli K* 12 strains *W* 1177 and 58/161, are distributed in manners which are sufficiently different to allow mixtures of the two strains to be separated by the counter-current procedure. *E. coli K* 12 strains of opposite sex do not seem always to have different distribution patterns, however. For example, as mentioned before, the *E. coli K* 12 female strain *C* 600 is distributed in a very similar fashion to the *E. coli K* 12 male strains 58/161 and *C*. A more detailed study of the behaviour of sex strains in aqueous polymer phase systems and of the influence of surface properties on the distribution of bacteria in these systems is in progress.

We wish to thank Prof. A. Tiselius for his interest in this work. Financial support has been received from the Department of Scientific and Industrial Research in the form of a research fellowship (to G. D. B.), and from the Swedish Technical Research Council.

[1] Craig, L. C., in *A Laboratory Manual of Analytical Methods of Protein Chemistry*, edit. by Alexander, P., and Block, R. J., **1**, 121 (Pergamon Press, Oxford, 1960).
[2] Albertsson, P. Å., *Partition of Cell Particles and Macromolecules* (Almqvist and Wiksell, Stockholm; John Wiley and Sons, Inc., New York, 1960).
[3] Albertsson, P. Å., and Nyns, Ed. J., *Nature*, **184**, 1465 (1959).
[4] Albertsson, P. Å., and Frick, G., *Biochim. Biophys. Acta*, **37**, 230 (1960).
[5] Albertsson, P. Å., and Nyns, Ed. J., *Arkiv Kemi*, **17**, 197 (1961).
[6] Raymond, S., *Anal. Chem.*, **30**, 1214 (1958).

EFFECT OF TEMPERATURE DURING GERMINATION ON THE CRYSTALLINE TYPE OF STARCH IN SOYBEAN SEEDLINGS

By Dr. SUSUMU HIZUKURI, Miss MICHIKO FUJII and Prof. ZIRO NIKUNI
Institute of Scientific and Industrial Research, Osaka University

THE crystalline structure of native starch granules is not identical, and the three main types, the *A*-, *B*- and *C*-types[1], are known. The *U*-type, which has been found recently in *Ulva expana* starch[2], is an exceptional type. The *A*-type has been found mainly in cereal starches, the *B*-type in tuber starches, and the *C*-type in bean and root starches. Some exceptional cases have been found as, for example, in amylomaize (*B*-type)[3], wrinkled pea (*B*-type)[4] and *Colocasia antiquorum* (*A*-type)[5]. The *C*-type is a transition type between the *A*- and *B*-types and many varieties are known, some resembling the *A*-type (*Ca*-type), some being intermediate (*Cc*-type) and some resembling the *B*-type (*Cb*-type). A transition series from the *A*-type (rice) to the *B*-type (potato) through various *C*-types has been observed by Nikuni *et al.*[6,7].

Some hypotheses have been proposed as to why these various structural modifications occur, but this important problem has not been elucidated. We tried to attack this problem by studying the effect of environmental conditions on crystallization, using amylodextrin (short-chain amylose) as test material[8,9]. It was shown that the crystalline type of amylodextrin obtained depends entirely on crystallization conditions such as the temperature of crystallization[9], the concentration of amylodextrin[9] and the kind and concentration of inorganic ions in the mother liquor[8].

In this article, the effect of temperature on the crystalline type of starch in living plants is reported.

Mature soybeans (*Glycine soja*) contain practically no starch, though starch is produced during the germination period to the extent of several per cent of the weight of the seedlings[10,11]. Therefore, seedlings germinated at different temperatures were very suitable material for this work.

Batches of 50 gm. of soybeans were soaked in water at various constant temperatures (10–40° ± 0·5° C.—the temperature-range at which germination is possible). They were kept in a thermostat at these same constant temperatures for 24 hr., or for 48 hr. when the temperature was below 13·5° C. Then the soaked beans were transferred to wet purified cotton beds in deep Petri dishes (12 cm. in diameter and 5 cm. deep). Beans were germinated in the dark, keeping the thermostat at the same fixed temperatures. It took 3–10 days, depending on the temperature, for seedlings to germinate. During incubation, the seedlings were washed with water at the various temperatures and transferred carefully and quickly once or twice a day to new beds to prevent bacterial infection and to provide sufficient humidity. The water content of the cotyledons was 59–64 per cent and that of hypocotyls was 88–92 per cent.

These parts were homogenized with cold water (about 5° C.) for 6 min. in a homogenizer. The homogenate was filtered through filter paper (Toyo Roshi No. 2), with suction. Starch granules were small enough to pass through the filter paper (0·3–3·5μ in diameter) and were thus separated from the cell debris. The residual cell debris were homogenized twice more in the same way. The resulting filtrates were combined and centrifuged at 3,500 r.p.m. for 5 min. The starch fraction, which sedimented, was washed repeatedly with cold water, and finally with methanol, and was then dried to about 20 per cent moisture content. The resulting starch preparation was examined in an X-ray diffractometer. The X-rays used were nickel filtered copper radiations.

The results showed that the crystalline type of the starch obtained from the cotyledons and hypocotyls of soybean seedlings varied greatly with the temperature at which germination took place. A transition series from the *B*-type to one very similar to the *A*-type (*Ca*-type) was obtained with elevation of the germination temperature. The *B*-type appeared at germination temperature below 13·5° C., the *Cb*-type at 16° C., the *Cc*-type at 22° C., and the *Ca*-type at or above 28° C. There was no difference in the type of the starch in the cotyledons and hypocotyls of the same seedlings.

To investigate a mixed type of starch, an incubation was carried out at alternating temperatures. Thus, soaked beans were kept at 32° C. for the first 10 hr. and then the Petri dish was transferred to a 13·5° C. thermostat for the next 14 hr. This altera-

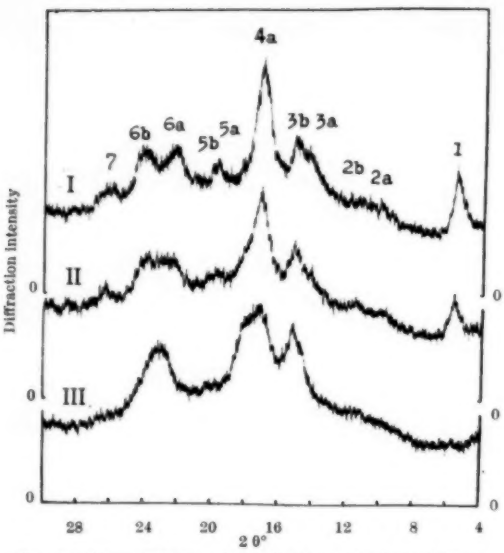


Fig. 1. X-ray diffractometer patterns of starch in soybean seedlings germinated at various constant temperatures. Germination temperature: I, 13·5° C. (*B*-type); II, 22° C. (*Cc*-type); III, 30° C. (*Ca*-type, faint 1-line seen)

tion of temperature was repeated five times during the 5-day period of incubation. The starch obtained from these seedlings was the *Cc*-type, which corresponded to that formed at the intermediate temperature of 22° C. This is further evidence for the idea in ref. 7 that the *C*-type is a mixture of the *A*- and *B*-types.

Typical X-ray diffractometer patterns are shown in Fig. 1. The relation between the diffractometer patterns and the temperature are exactly reproducible, in the same batch of soybeans. Patterns of starch from younger and older seedlings at the same temperature were identical.

This relation between the crystalline type of native starch and the germination temperature coincided with the relation between the crystalline type of amylodextrin and the temperature of crystallization which was reported in ref. 9.

These facts indicate that the crystalline type of native starch is not always decided by the botanical classification of the plant and that it is very much affected by the environmental conditions during the formation of the starch. In fact, some variations in crystalline type have been found in peas[12], mung beans (*Phaseolus aureus*)[13] and sweet-potatoes[14].

[1] Katz, J. R., and van Itallie, Th. B., *Z. physik. Chem.*, A **150**, 90 (1930).
[2] Meeuse, J. D., and Kreger, D. R., *Biochim. Biophys. Acta*, **35**, 26 (1959).
[3] Dvonch, W., Kramer, H. H., and Whistler, R. L., *Cereal Chem.*, **28**, 270 (1951).
[4] Hilbert, G. E., and MacMasters, M. M., *J. Biol. Chem.*, **162**, 229 (1957).
[5] Fujii, M., Hizukuri, S., and Nikuni, Z. (unpublished work).
[6] Nikuni, Z., Fuwa, H., and Tatsumi, C., *J. Agric. Chem. Soc.*, Japan, **22**, 91 (1948).
[7] Hizukuri, S., and Nikuni, Z., *J. Agric. Chem. Soc.*, Japan, **31**, 525 (1957).
[8] Hizukuri, S., Fujii, M., and Nikuni, Z., *Biochim. Biophys. Acta*, **40**, 346 (1960).
[9] Hizukuri, S., *Agric. Biol. Chem.* (Japan), **25**, 45 (1961).
[10] Sasaki, S., *J. Agric. Chem. Soc.*, Japan, **5**, 491 (1929); **9**, 693 (1933).
[11] Von Ohlen, F. W., *Amer. J. Bot.*, **18**, 30 (1931).
[12] Badenhuizen, N. P., *Protoplasmatologia, Handbuch der Protoplasmaforschung*, **2**, 12 (1959).
[13] Kawamura, S., and Hizukuri, S., *Techn. Bull. Facul. Agric.*, Kagawa Univ., **11**, 234 (1959).
[14] Hizukuri, S., and Nikuni, Z. (unpublished work).

INDUCTION OF A LIGHT REQUIREMENT FOR THE GERMINATION OF LETTUCE SEED BY NARINGENIN, AND ITS REMOVAL BY GIBBERELLIC ACID

By Dr. I. D. J. PHILLIPS

Commonwealth Scientific and Industrial Research Organization, Irrigation Research Station, Griffith, New South Wales

NARINGENIN (5,7,4′-trihydroxyflavanone) has recently been isolated from dormant buds of peach[1], with the suggestion that it is a plant growth-inhibitor playing a functional hormonal role in the control of dormancy[2]. I have been examining some effects of naringenin on several growth and dormancy systems, during the course of which it has become apparent that naringenin exhibits certain properties very similar to that of coumarin, another well-known naturally occurring plant growth-inhibitor.

One of the most interesting effects of coumarin on plants is the observation that it can induce a light requirement for germination in varieties of lettuce that are normally not light-requiring[3], and that such an induced requirement can be removed by gibberellic acid[4]. Thus it was decided to investigate the possibility that naringenin, too, could induce a light requirement by non-dormant lettuce seed, despite its structure being very different from that of coumarin.

Commercially supplied lettuce seed (var. Great Lakes) was used in the following experiments. The seeds were allowed to germinate in either darkness or light in 9 cm. diameter Petri dishes on Whatman 1/64 in. seed-test papers, which had been moistened with either 4 ml. distilled water in the controls, or with 4 ml. test solution. Light was supplied by a bank of 'Daylight' fluorescent tubes 88 cm. above the Petri dishes, and the temperature was maintained at a constant 21° ± 1° C. Germination was allowed to proceed for 48 hr., at the end of which time the percentage germination per treatment was determined. There were approximately 300 seeds in each treatment.

Germination of seeds in a range of aqueous naringenin concentrations revealed that, up to 20 mgm./l. naringenin, there was no inhibition of germination

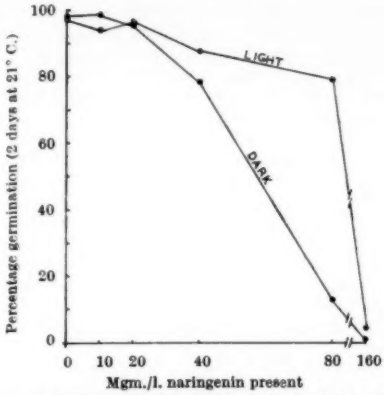


Fig. 1. Result of germinating seeds of Great Lakes lettuce (approximately 300 seeds per treatment) at 21° ± 1° C., in aqueous solutions of naringenin, or in water. Open points, seeds allowed to germinate in light; closed points, seeds germinated in darkness

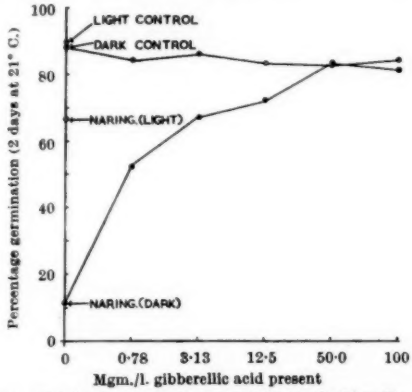


Fig. 2. Effect of a range of concentrations of gibberellic acid on the dark-inhibiting effect of an 80 mgm./l. solution of naringenin, on the germination of Great Lakes lettuce seed. ○, gibberellic acid alone; ●, gibberellic acid plus naringenin

in either light or dark, but that at 40 mgm./l., and particularly at 80 mgm./l. there was a suppression of germination, with much more inhibition in darkness than in light (Fig. 1). This result has been fully confirmed in other experiments. Consequently naringenin behaves similarly to coumarin in this respect.

The effect of a range of gibberellic acid concentrations on the dark germination of seeds in a solution of 80 mgm./l. naringenin was measured, and the result shown in Fig. 2. It can be seen that gibberellic acid reverses the light requirement induced by naringenin, and that the extent of the reversal is a function of the ratio of the number of gibberellic acid molecules present to molecules of naringenin. It might be said, therefore, that there is a competitive relationship between gibberellic acid and naringenin in their effects on lettuce-seed germination in darkness. This again is similar to what has been found for coumarin[4].

It is interesting that two such chemically dissimilar compounds as coumarin and naringenin have the same effects on lettuce-seed germination, and that these effects can be counteracted by gibberellic acid. However, both compounds are naturally occurring plant-growth inhibitors, and there is an accumulation of evidence that such substances may be active in the control of dormancy. It is conceivable that both are active as inhibitors of growth in dormant tissues by acting as competitive inhibitors of endogenous gibberellin of the tissues. This hypothesis is supported by the evidence that gibberellins are present in plant tissues[5], and that inhibitors are found particularly in dormant organs. However, in work on auxin-antagonists it has generally been found that where such a compound competitively inhibits auxin then the 'competition' for sites of action between antagonist and auxin can be related to chemical structure[6]. Perhaps in the case of coumarin and naringenin there is a common basis in their molecular structures for their similar physiological effect, but elucidation of what that basis is must wait until the effects of other compounds on dormancy systems are investigated to enable a closer comparison to be made.

It is possible that such apparent competitive inhibition of gibberellic acid by either coumarin or naringenin as reported here may not be a true, simple competition of molecules for 'sites of action', as is the case for competitive enzyme inhibitors. If this were so, then it might be that a wide variety of divergent types of compound will behave similarly to coumarin and naringenin, with no common structural basis to explain the similarity. However, it has been reported[7] that any change in the molecular structure of coumarin, as brought about by substitution, will cause a decrease in the inhibiting effect of the molecule. This would seem to indicate that there is some specific molecular requirement for inhibitory activity.

[1] Hendershott, C. H., and Walker, D. R., *Science*, **130**, 3378, 798 (1959).
[2] Hendershott, C. H., and Walker, D. R., *Proc. Amer. Soc. Hort. Sci.*, **74**, 121 (1959).
[3] Nutile, G. E., *Plant Physiol.*, **20**, 433 (1945).
[4] Mayer, A. M., *Nature*, **184**, 4689, 826 (1959).
[5] Phinney, B. O., and West, C. A., *Ann. Rev. Plant Physiol.*, **11**, 411 (1960).
[6] McRae, D. H., and Bonner, J., *Plant Physiol.*, **28**, 243 (1953).
[7] Mayer, A. M., and Evenari, M. J., *J. Exp. Bot.*, **3**, 246 (1952).

CHLOROGENIC ACID: AN ALLERGEN IN GREEN COFFEE BEAN

By Dr S. O. FREEDMAN, J. KRUPEY and A. H. SEHON

McGill University Clinic of the Montreal General Hospital and Departments of Biochemistry and Chemistry, McGill University

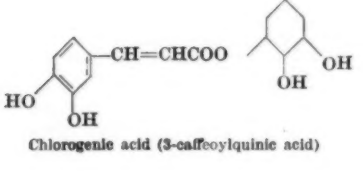
IT is well known that workers in the coffee industry frequently develop bronchial asthma, rhinitis or dermatitis on exposure to the fine dusty powder which fills the air of the factory as the chaff is mechanically stripped from the raw bean prior to roasting. Previous investigations[1] showed that all workers so affected gave large positive immediate type intradermal reactions to aqueous extracts of green coffee chaff and green coffee bean. Skin-sensitizing antibody (reagin) to green coffee was shown to be present

in the sera of these allergic workers by passive transfer (Prausnitz–Kustner) experiments. Extracts of roasted coffee, on the other hand, gave minimal or negative reactions on both direct and passive transfer testing.

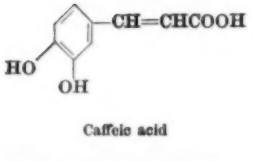
Chlorogenic acid, a compound of quinic and caffeic acids, is one of the major water-soluble constituents of green coffee, accounting for 5–8 per cent of the dry weight of the bean[2]. It was first obtained in crystalline form from green coffee beans by Gorter[3]. On the basis of a ferric chloride colour reaction, he concluded that this compound was widely distributed in the leaves and seeds of numerous plants; but it is now known that Gorter's test is not specific for chlorogenic acid since ferric chloride will give a colour reaction with most phenolic compounds. Nevertheless, chlorogenic acid has been shown to be present in significant quantities in apples, pears, carrots, beans, celery, lettuce, cucumber, cabbage, plums, cherries, sweet potatoes and tobacco[4–8]. Joslyn and Ponting have suggested that the darkening of certain cut fruits on exposure to air is due to the oxidation of chlorogenic acid to a quinone, rather than to the oxidation of true tannins[9].

The purpose of the present work was to identify the chemical nature of the allergen(s) present in green coffee bean. The aqueous extract investigated was highly allergenic, producing a markedly positive wheal and erythema response on direct skin testing with 0·02–5 ml. of a solution containing 5 mgm./ml. and a positive passive transfer reaction at a serum dilution of 1 : 3,200.

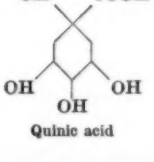
The aqueous extract of green coffee was fractionated by chromatography on dextran gel ('Sephadex *G*-25', purchased from Pharmacia, Uppsala). Forty-five fractions were collected and tested for allergenicity by the passive transfer method. The middle fractions (18–29) gave strongly positive passive transfer reactions, while the remainder of the fractions gave minimal or negative reactions. All the allergenically active fractions were coloured brown and gave a green colour reaction with ferric chloride, while the allergenically inactive fractions were colourless and gave a negative ferric chloride test. These observations suggested that phenolic compounds were present in much higher concentrations in the allergenically active fractions.



Chlorogenic acid (3-caffeoylquinic acid)



Caffeic acid



Quinic acid

A systematic evaluation of the known phenolic compounds in coffee was undertaken by injecting the suspected compounds into the skins of ten workers known to be allergic to green coffee. Only chlorogenic acid produced positive reactions. 1 μgm. of chlorogenic acid was sufficient to induce a large wheal and flare response with pseudopods in all the subjects tested. On passive transfer testing with the sera obtained from five of these workers, as little as 5×10^{-5} μgm. of chlorogenic acid was required to elicit a reaction at a site sensitized with 0·05 ml. of serum. Identical results were found with chlorogenic acid obtained commercially and with chlorogenic acid isolated from the aqueous extract of green coffee by the method of Moores *et al.*[3].

Cross-neutralization tests were performed with chlorogenic acid and an aqueous extract of green coffee in order to elucidate the allergenic relationship between these two substances. In one series of tests, sites in the skin of normal human recipients were passively sensitized with sera obtained from individuals allergic to green coffee. These sites were then challenged in 24 hr. by the intradermal injection of 1 μgm. of chlorogenic acid. On the following day, the same sites were re-challenged with 100 μgm. of green coffee extract. There were no reactions on re-challenge, indicating that all the antibody directed against the green coffee had been previously neutralized by the chlorogenic acid (Table 1).

Table 1. Results of Cross-neutralization Experiments

Serum from patients sensitive to green coffee	Initial reactions on passive transfer to 1 μgm. of chlorogenic acid	Reactions on rechallenge with 100 μgm. of green coffee
P. S.	+ + + +	–
P. S.	+ + +	–
A. S.	+ + + +	–
M. C.	+ + +	–
W. M.	+ + +	–

Complete cross-neutralization was also obtained by another technique. Chlorogenic acid and the serum of an individual allergic to green coffee were mixed *in vitro* in previously determined neutralizing proportions. 24 hr. later, this mixture was injected into the skin of a normal recipient. Challenge of this same site on the following day with green coffee extract produced no reaction. These results would suggest that chlorogenic acid (or chlorogenic acid complexed with other constituents of green coffee) may be the principal allergen in green coffee.

In an attempt to identify the allergenically active portion of the chlorogenic acid molecule, passive transfer tests were performed with caffeic and quinic acids. No positive reactions were obtained. However, when a passive transfer site previously sensitized with allergic serum was injected with a mixture of caffeic and quinic acids in equimolar proportions at a relatively high concentration (300 μgm./ml. and 320 μgm./ml. respectively), and the same site was re-challenged in 24 hr. with 1 μgm. of chlorogenic acid, the expected reaction was partially inhibited. Similarly, when a solution of quinic and caffeic acids at the same high concentrations was mixed with allergic serum prior to injection, there was partial inhibition of the passive transfer reaction to chlorogenic acid.

In order to explore the possibility of developing an *in vitro* test for precipitating or hæmagglutinating antibodies to green coffee, rabbits were immunized with a mixture of green coffee extract and Freund's adjuvant. A positive precipitin test was obtained when the rabbit antisera were treated with green coffee extract or with a solution containing both chlorogenic acid and human serum albumin. In the

hæmagglutination procedure, green coffee extract was linked to rabbit erythrocytes using the *bis*-diazotized benzidine technique described by Gordon, Rose and Sehon[10]. Hæmagglutinating antibodies against green coffee were detected in the rabbit antisera up to a dilution of 1 : 1,000,000. Chlorogenic acid, when used in the standard hæmagglutination procedure, produced non-specific hæmagglutination of rabbit erythrocytes and was therefore unsuitable for investigation by this method. The hæmagglutination reaction with green coffee was completely inhibited by the whole extract and by the previously described allergenically active coloured fractions obtained on chromatography.

No precipitation was obtained with human allergic sera, but one of the human sera so far tested was found to contain hæmagglutinating antibodies to green coffee up to a dilution of 1 : 1,280.

In conclusion, it would appear that chlorogenic acid may be the principal allergen in green coffee. The fact that an immediate skin reaction can be produced by a compound of low molecular weight would suggest that chlorogenic acid acts as a hapten in human skin or mucous membranes. A simple phenolic compound of low molecular weight has thus been identified as a major allergenic component of a plant material responsible for human atopic disease. Because polyphenolic compounds are widely distributed throughout the plant kingdom, it is possible that these observations may be useful in the search for the allergenic constituents of other foods, pollens, dusts or moulds.

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[1] Kaye, M., and Freedman, S. O., *Canad. Med. Assoc. J.*, **84**, 469 (1961).
[2] Moores, R. G., McDermott, D. L., and Wood, T. R., *Anal. Chem.*, **20**, 620 (1948).
[3] Gorter, K., *Arch. Pharm.*, **247**, 184 (1909).
[4] Rudkin, G. O., and Nelson, J. M., *J. Amer. Chem. Soc.*, **69**, 1470 (1947).
[5] Hulme, A. C., *J. Exp. Bot.*, **2**, 298 (1951).
[6] Hughes, J. C., *Chem. and Indust. Abstr.*, 214 (1958).
[7] Weaving, A. S., *Chem. and Indust. Abstr.*, 216 (1958).
[8] Hermann, K., *Naturwiss.*, **43**, 109 (1956).
[9] Joslyn, M. A., and Ponting, J. D., *Adv. Food Res.*, **3**, 1 (1951).
[10] Gordon, J., Rose, B., and Sehon, A. H., *J. Exp. Med.*, **108**, 37 (1958).

RESPONSE OF CHLOROPLAST CYTOCHROMES TO LIGHT AND SUBSTRATES

By Prof. H. LUNDEGÅRDH

Penningby, Sweden

THE introduction of a new spectrophotometric technique for investigation of oxidation-reduction processes in living material has promoted our knowledge of the respiratory enzymes in plants, for example, roots of cereals, and yeast[1-4]. The presence and activity of a number of cytochromes, flavoproteins and enzymes linked to pyridine nucleotide (PN) has been demonstrated in live cells and in mitochondria and observations of the steady-state changes at variation of the oxygen supply are revealing certain general features of the structural organization of the respiratory unit[3,4].

Chloroplasts, too, contain cytochromes, which are spectroscopically very similar to those in mitochondria[5]. Hill and Scarisbrick[6] found in chloroplasts a cytochrome *f*, characterized by an α-band at about 554 mμ and a prosthetic group similar to that of cytochrome *c*. Its spectral bands are very close to those of cytochrome c_1 in mitochondria. The cytochromes *c* and c_1 are predominantly oxidized in normally respiring mitochondria and are reduced rapidly in the absence of oxygen. Cytochrome *f* remains partially reduced in the dark and the α-band at 554 mμ may be observed easily in de-chlorophyllized chloroplasts. I showed in 1954 that it is oxidized if the chloroplasts are exposed to light[5]. Similar observations of the oxidation by light of a cytochrome of *c*-type were made in a number of photosynthetic micro-organisms[8]. During my work on the response of cytochrome *f* to light I also observed partly opposite reactions of a cytochrome *b* (ref. 5). Hill reported[9] the presence of a cytochrome b_4 with an α-peak at about 563 mμ. Finally, a cytochrome b_3 was distinguished with an α-peak of the reduced enzyme at about 559 and a γ-band at 429 mμ (ref. 10). This cytochrome is very similar to a cytochrome b_3, which is probably a regular constituent of the respiratory unit in plant tissues. Very little has hitherto been reported regarding an eventual response of the *b*-group in chloroplasts to light. The whole problem thus called for a re-investigation, particularly in connexion with the recent discoveries regarding photophosphorylation.

Arnon[11] first showed the actual existence of a light-induced phosphorylation in chloroplasts, linked, in his results, with the mentioned interaction of cytochromes in the light response. According to Arnon's scheme of photophosphorylation, chlorophyll converts light-energy in a stream of electrons which are passing through a cycle of redox-enzymes, among these, cytochromes. The cytochromes in chloroplasts are figured co-operating in phosphorylation in much the same way as in mitochondria.

Chloroplasts also produce reduced pyridine nucleotide[11,12] (PN, preferably triphosphopyridine nucleotide (TPN)) and, simultaneously, oxygen. It is commonly assumed that PN is reduced by the electrons expelled from the excited chlorophyll plus protons (H^+) from water. The remaining OH^- ions may lose their electrons to the cytochrome system and simultaneously produce oxygen and water. Water thus acts as a bridge connecting the reducing (Chl. → TPNH) and oxidizing (cytochromes → Chl.) moieties of the cycle. The net effect of the water bridge is to move electrons in the direction of the potential gradient and protons in the opposite direction. The formation of adenosine triphosphate (ATP) and reduced pyridine nucleotide (PNH) takes place in the pigmented grana[13]. The reduction of $CO_2 + H_2O$ to carbohydrate (CH_2O) is a dark chemo-synthetical

process which takes place in the non-coloured stroma of chloroplasts.

In continuing my work on the response of chloroplast cytochromes to light I have kept in mind the results of my recent investigations on steady-state relations of the respiratory enzymes in baker's yeast[14]. It was shown that the intensity of respiration and the apparent velocity of reduction of the involved cytochromes are synchronously lowered on the addition of ATP, and raised on the addition of ADP + PO_4. This is what would be expected if the steady-state balance ADP + PO_4 ⇌ ATP were moved in one direction or the other on changing the concentration of the substrates, and if the process of phosphorylation serves as an efficient path of electron transfer between two cytochromes. A displacement of the steady-states of pyridine nucleotides may also have an influence. Because in chloroplasts both pyridine nucleotides and adenosine phosphates are directly or indirectly involved in the transfer of electrons between chlorophyll and water it may be expected that a rise of concentration of one of these substrates will exert a significant influence on the light response.

Cultivated and wild plants, including spinach, wheat, clover (*Trifolium repens*), *Agrostis stolonifera* and *Chenopodium album* served as experimental material. The leaves were ground with sand and 0·3 *M* sodium chloride with 0·02 mol. *tris* buffer and the brei squeezed through double cheese-cloth. For clover and spinach homogenization for 5–7 sec. also gave good results. Coarse particles were removed by centrifugation for 1 min. at 100–200*g*. The supernatant was spun for 7 min. at 1,400*g* and the pellet, containing the whole chloroplasts, was washed and re-centrifuged in 0·3 *M* sodium chloride. All manipulations, including the spectrophotometric observations, were carried out at approximately 0° C. The final suspension in 0·3 *M* sodium chloride contained about 30 mgm. chloroplasts (dry weight) in 10 ml. solution (0·3 per cent).

For the spectrophotometric analysis 10-mm. cuvettes were provided with an inset of gilded brass leaving a central 2-mm.-wide slit. The cuvettes were placed in a holder about 30 mm. from the entrance slit of a Bausch and Lomb monochromator. The enclosed portion of the suspension (2 × 10 × 10 mm.) was illuminated by focusing on it the straight band of a tungsten lamp (through a thick plate of heat-absorbing glass). By means of a suitable shutter the light was turned on immediately after the start of the spectrophotometric recorder. Two instruments of my own construction were used for this investigation. One instrument moves the wave-length screw 2 mμ, in intervals of 0·33 or 0·5 sec. and yields a record (high-speed pen recorder) of the region 540–570 mμ in 5·0–7·5 sec., which is repeated in intervals of 10 sec. The second instrument is of the scanning type and moves the wave-length screw 540–570 mμ in 0·25 sec. with immediate repetition of the movement. An oscillographic recorder yields 180 complete spectrograms in 1 min.

Occurrence and Percentage of Cytochromes in the Chloroplasts

Because the state of oxidation-reduction of the cytochromes is varying according to light intensity and availability of co-factors and substrates, measurements of the amplitudes of the reduced α-bands under varying conditions are revealing not only the

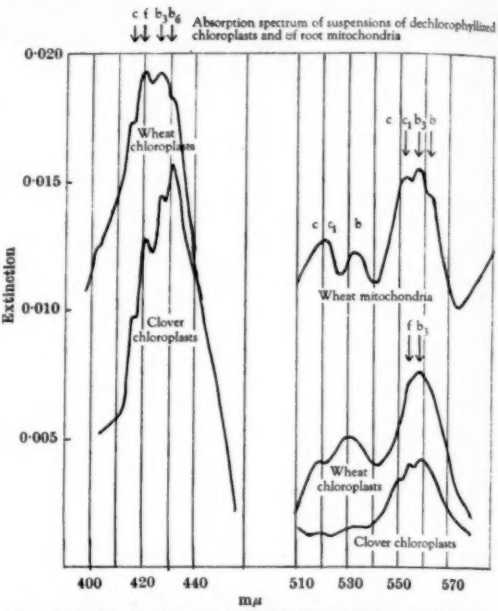

Fig. 1. Reduced cytochromes (dithionite) in chloroplasts of wheat and clover, from which the chlorophyll was removed by treatment with alcohol, compared with cytochromes in mitochondria

types of involved cytochromes, but also the reacting quantities. Direct spectrograms of chloroplast suspensions or whole leaves show band-peaks or elevations at 550, 554, 556–558 and 562–564 mμ, corresponding to cytochromes *c*, *f*, b_3 and b_6. The close relation of cytochrome b_3 to a corresponding cytochrome in roots and of b_6 to the cytochrome *b* in mitochondria appears from records of the γ-region of de-chlorophyllized chloroplasts in dithionite (Fig. 1). Peaks are appearing here at 416 (*c*), 420 (*f*), 426–427 (b_3) and 430–432 mμ (b_6). The relation in height between the γ-bands and the α-bands amounts to 4–7, or about the same as in wheat roots and in preparations of cytochromes *c*, *f*, c_1 and *b*.

After removal of the chlorophyll with alcohol and re-suspending in 0·3 *M* sodium chloride, chloroplasts and whole leaves show a reduced band of cytochrome *f* which is further increased in dithionite. The weak bands at 416 and 550 mμ are not always clearly recorded, and the possibility cannot be *a priori* excluded that contaminations from protoplasm are at work. A comparison between the spectrograms from whole leaves and from suspensions of chloroplasts does not support this possibility, however, and it must be concluded that chloroplasts contain certain quantities of a cytochrome, the bands of which lie close to those of cytochrome *c*. The complete sets of cytochromes in chloroplasts and mitochondria are thus surprisingly similar to one another even if the proportions are different (see Fig. 1). Besides the above-mentioned differences, roots contain about 2–4 times as much cytochrome *b* as cytochrome c_1, whereas in wheat chloroplasts the relation b_3 to *f* is about 1, and b_6 is less abundant than *b* in plant mitochondria. A suspension of chloroplasts of clover contains 3–5 times as much cytochrome as a yeast suspension of equal concentration.

For calculation of the percentage of cytochrome in chloroplasts, measurements were made at the follow-

ing wave-length intervals: 550–542, 554–542, 558–542, or 558–570 and 564–570 mμ. The calculations are approximate, primarily because of the partial overlapping of the bands of *c* and *f* and of *f* and b_3. About 25 per cent of the α-band of *f* covers the α-band of b_3.

In 0·3 per cent chloroplast suspensions the amplitude of variations in the state of oxidation-reduction in maximum amounted to about $\varepsilon = \pm 0{\cdot}03$, corresponding to about 2·5 per cent cytochrome ($c + f + b_3 + b_6$) calculated per unit dry weight. Leaves of clover contain higher quantities than those of wheat seedlings. It was assumed that the group $c + f$ has a molecular weight of 15,000 and the group $b_3 + b_6$ 30,000 (ref. 15). These calculations are, of course, only tentative. De-chlorophyllized chloroplasts yield lower values, or 0·7 per cent, probably because some cytochrome is extracted or disorganized in alcohol. Alcohol also removes most of the lipids, which, together with chlorophyll, contribute about 50 per cent of the solid substance of the chloroplasts. Per unit of iron, group *c*–*f* and the group b_3–b_6 have about equal percentages of the total quantity of cytochromes.

For comparison, determinations were also made on mitochondria from wheat roots, yielding 0·5–1·0 per cent cytochrome per unit dry weight (earlier figures are somewhat higher, because it was then calculated with a molecular weight of cytochrome *b* = 60,000). We thus arrive at the conclusion that chloroplasts contain 2–3 times more cytochrome than mitochondria from the same plant. The observed rapid interaction of chlorophyll and cytochromes, and the fact that the grana are the sites of photophosphorylation, support the conclusion that all cytochromes are structurally linked to chlorophyll.

Response to Light

According to current opinions (see above) the excited chlorophyll maintains a flux of electrons through a cycle of redox enzymes and co-factors making up an internal circuit and, if $H^+ + e^-$ is consumed, water the external circuit. Warburg discovered [16] that chloride was acting as a co-factor. Chloride ions may here possibly act in a similar way to salt ions in respiration, in other words facilitating the shuffling of electrons over a water bridge. In photosynthesis the chlorophyll may be thought of as operating in the same way as the cathode of a photocell, expelling electrons in light, like many other dyes[17]. The conversion of electrical energy into chemical energy, namely, the synthesis of reduced pyridine nucleotide and high-energy phosphate, occurs at a few intermediate points.

The structural organization of the respiratory unit of mitochondria is mirrored in a rhythmic course of reduction at removal of the oxygen[2,3]. Reduction invariably starts with the enzymes of highest oxidation potential (cytochrome oxidase (a_3) and cytochrome *c*). After a gap of 1–2 sec. (in yeast at room temperature) or a few minutes (in wheat roots) reduction of cytochrome *b* begins. Observations of the time course of reduction by means of rapidly recording spectrophotometers[4] thus serve as a useful tool for investigating the individual response of the single respiratory enzymes to environmental influences. At re-oxidation the time course is reversed, now starting at the enzymes of the highest reduction potential, but the rhythm is here less clearly observed, because re-oxidation, as a rule, proceeds very quickly.

Chloroplasts have no cytochrome oxidase and do not respire[11]. Their electronic state in darkness thus corresponds to that of mitochondria which lack oxygen. Light converts chlorophyll into an oxidase which attracts electrons from the adjacent cytochrome *f*. Electrons are now flowing through the whole cytochrome system and continuously replaced by those expelled from the excited chlorophyll. Each link in the cycle is steered by an on-reaction and an off-reaction $\left(\frac{e^-}{\text{on}} \rightarrow \text{cyt.} \rightarrow \frac{e^-}{\text{off}}\right)$ and the single enzymes, at least some of them, are interspersed by speed-regulating 'factors'. A close observation of the time course might also reveal an individual response: I have in this article restricted myself to observing the sign and magnitude of the response of the cytochromes.

Recent investigations of Witt and co-workers[18] have shown that the immediate activation of chlorophyll may be observed a few milliseconds after turning on the light and also that activation of the water bridge probably starts very rapidly. My own oscillographic records show, in less than 0·1 sec., a rapid rise in extinction of the whole green trough between 540 and 570 mμ. The extinction is highest in the region 555–560 mμ, but this broad band does not belong to a cytochrome. A possible reactant is catalase. The role of catalase in chloroplasts has been widely discussed, but we cannot go into this problem here. The possibility of catalase guiding the de-ionization of OH^- and transference of electrons to cytochrome b_3 deserves serious consideration.

The response of the cytochromes to light is considerably slower than the initial reaction already mentioned. In my first publication the response was measured about 10 sec. from the start[5]. New records in time-intervals of 0·3–0·5 sec. afforded values down to 2 sec. but most of them centred around 6–8 sec.

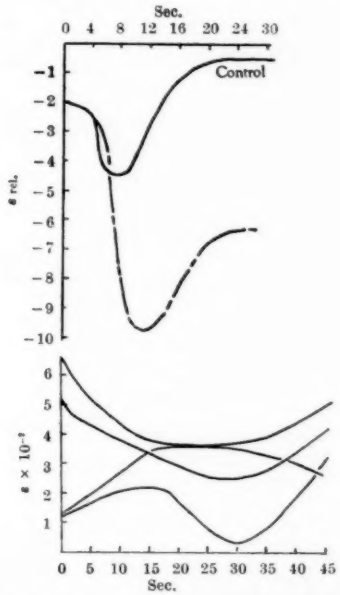

Fig. 2. Above, the response of cytochrome *f* to light. Washed chloroplasts of *Chenopodium album*. Upper curve in 0·3 *M* NaCl, lower curve 0·3 *M* NaCl + 0·015 *M* ADP as sodium salt. Below, response of chloroplast cytochromes of *Trifolium repens* to light. The light was in both cases turned on at 0 sec.

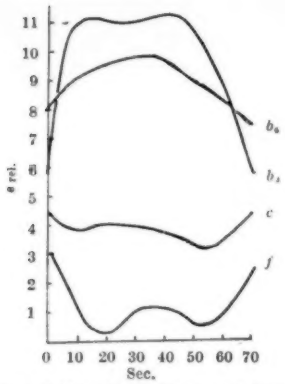

Fig. 3. Response of chloroplast cytochromes of *Agrostis stolonifera* to light. Whole leaves. The light was turned on at 0 sec.

(Fig. 2). The existence of a starting lag was observed in all plants, both in whole leaves and in chloroplast suspensions. In a number of cases different starting lags were observed regarding cytochromes *f* and b_3. The oxidation of cytochrome *f* in light started earlier than the oxidation of cytochrome b_3 (and b_6) by cytochrome *f*. This result refers to washed chloroplasts and shows a more rapid supply of electrons from TPNH to b_3 and from *f* to chlorophyll than from b_3 to *f*, when phosphorylation is retarded for lack of substrates.

Similar to the length of the starting period, the time course of the response to light may vary too. All these observations were made on chloroplast suspensions in 0·3 *M* sodium chloride. Ageing suspensions sometimes react differently to light and may show either an insignificant response or an increased reduction of the cytochromes instead of oxidation (Figs. 4 and 5). Sometimes cytochromes *f* (and *c*) react in an opposite way to the group b_3–b_6. This counter reaction of the *b*-group, which was observed also in 1954 (ref. 5), is shown in Fig. 3. These observations also show a lowering of the conductivity between the *b*-group (primarily b_3) and the *f* (*c*)-group, a fact reminiscent of the relation between the cytochromes *b* and *c* in mitochondria, which is exposed to a number of influences, for example, inhibition by urethane and antimycin *A* (refs. 1 and 19).

Variations in the response to light are always to be expected from a cyclic system in which the individual on- and off-reactions are regulated by interaction with different enzymes or 'factors'. Some of the intermediate factors have been tested, for example, flavin adenine mononucleotide (FMN), vitamin K, etc.[11].

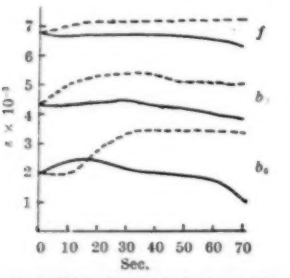

Fig. 4. Response to light of unwashed wheat chloroplasts after addition of ferricyanide (10 mgm. in 1 ml.). The controls (broken curves) show in this experiment an increased reduction of the cytochromes after turning on the light (see text)

These co-factors are controlling the supply of electrons to the cytochrome system. My experiments show that addition of FMN turns a negative response to light (oxidation of cytochromes), into a positive one (reduction in light). This may be explained as follows: FMN lowers the resistance to internal transport of electrons from excited chlorophyll to the cytochromes, a result in agreement with observations on photophosphorylation[11]. An opposite effect —an increase of the light-induced oxidation of the cytochromes—is observed on addition of ferricyanide. This substance is obviously trapping the electrons before they reach the cytochromes, but does not impede the reduction of chlorophyll (Fig. 4).

Influence of Substrate Factors on the Light Response

In these experiments sodium salts of DPN or DPNH (Sigma; acting similar to TPN and TPNH, but slightly weaker) and ATP or ADP were added in quantities corresponding to 0·01–0·02 *M*. After a period of 10–60 min. in the dark, the response to light was recorded as above. For determination of the dark response to the substrates the recipients were placed in front of the photomultiplier tube. The weak light from the monochromator does not appreciably disturb the steady states.

As shown in Table 1, addition of DPN induces increased oxidation of the cytochrome system. The opposite effect of DPNH is notably observed as a reduction of cytochrome b_3. The weak response of cytochrome *f* to reducing agents is due apparently to its normally predominant reduction in the dark. The results indicate the existence of a steady state between pyridine nucleotide and cytochromes, in which transferring co-factors (FMN, etc.) may be involved, according to the scheme in Fig. 6 and reaction 1 (see later). A rise in the concentration of PN moves the steady state in one direction; addition of DPNH promotes the reverse reaction. The pronounced response of b_3 points to this cytochrome functioning as the switch-board between the reducing (Chl. → PN → co-factors) and the oxidizing moieties (cytochromes → Chl.) of the cycle.

Table 1. Dark Reactions: Effects of Substrates on the State of Oxidation-Reduction of the Cytochromes. Chloroplasts from Wheat Seedlings

Cytochromes	*f* (+*c*)	b_3	b_6
+0·015 *M* DPN	Oxidation	Oxidation	Oxidation
+0·015 *M* DPNH	Weak reduction	Strong reduction	Weak reduction
+0·015 *M* ADP(+PO_4)	Weak oxidation	Weak oxidation	Weak reduction
+0·015 *M* ATP	Reduction	Reduction	Reduction

As to the adenosine phosphates, recent observations on yeast showed that a supply of ADP + (PO_4) accelerates the electron transference, notably from cytochrome *b* to *c* (and c_1), whereas addition of ATP retards it. The effects were also observed as an increase or a decrease of the intensity of oxygen consumption. It was concluded that variations in the mutual concentration of ADP and ATP cause the steady-state balance ADP + (PO_4) ⇌ ATP to move forwards or backwards.

In the dark chloroplasts the chlorophyll remains saturated with electrons and cannot absorb more from cytochrome *f*. This cytochrome consequently remains reduced to a degree which is determined by the total oxidation–reduction balance between PN, PNH, co-factors, and cytochromes. Substrate influences will thus, to a certain degree, be reflected in a displacement of the corresponding steady states

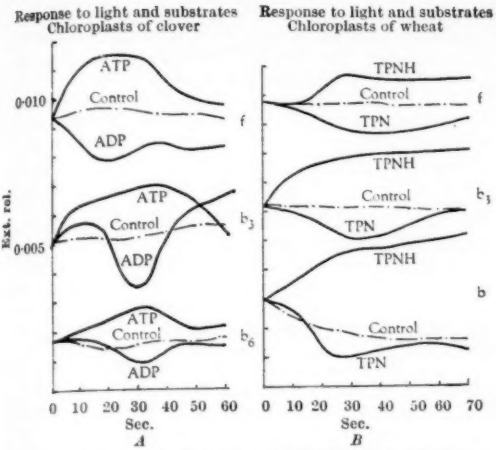

Fig. 5*A*. Response to light of washed chloroplasts of clover (*Trifolium repens*) in 0·3 *M* NaCl after addition of ATP or ADP (8 mgm. in 1 ml.). Exposure (at 0 sec.) made 40 min. after addition of substrates. Controls (broken curves) in 0·3 *M* NaCl alone

B. Response to light of washed chloroplasts of wheat in 0·3 *M* NaCl after addition of TPN and TPNH (as sodium salts; 6 mgm. in 1 ml.). Controls (broken curves) in 0·3 *M* NaCl alone

in the dark. The response of cytochromes to ADP or ATP in the dark (Table 1) indicates the position of a point 2 of phosphorylation between b_3 (and/or b_6) and f (and/or c). Changes in the steady state of b_3 and b_6 indicate a point 3 of phosphorylation between these cytochromes (or one of them) and the dehydrogenizing co-factors (see also Fig. 6).

The response to light (Table 2 and Fig. 5) illustrates a further development of the dark effects; the reactions are still more intensified except in the case of b_6, which responds to ADP or ATP much in the same way as in respiring yeast. The rising oxidation of yeast cytochrome *b* on the addition of ATP was interpreted as a retardation of the stream of electrons from dehydrogenases to cytochrome *b*. This conclusion may be applied also to the chloroplasts. The increased reduction of b_3 of chloroplasts on the addition of ATP indicates the existence of a point 2 of phosphorylation between b_3 and f, the inhibition of which retards the off-reaction of b_3 and consequently promotes its reduction. The fact that cytochrome *f* is less oxidized in light after addition of ATP is interesting because it indicates the existence between *f* and Chl.$^+$ of a point 1 of phosphorylation, which is absent in the transference $a_3 \rightarrow O_2$ in the respiratory chain.

Table 2. Response to Light: Average Effects of Substrates on the Light-reaction of Chloroplasts of Clover and Wheat
Period of illumination, 60 sec. Spectral records at short intervals. Controls without organic substrate showed weak oxidation

Cytochromes	$f(+c)$	b_3	b_6
+0·015 *M* DPN	Oxidation	Oxidation	Weak oxidation
+0·015 *M* DPNH	Weak reduction	Reduction	Reduction
+0·015 *M* ADP(+PO_4)	Oxidation	Oxidation	Weak oxidation
+0·015 *M* ATP	Weak oxidation	Reduction	Oxidation

Discussion

The new experiments corroborate my work in 1954 regarding oxidation of cytochrome *f* in light[5]. I claimed that "it is feasible to assume that radiant energy transformed into chemical energy may oxidize cytochrome *f* or alternatively a special cytochrome system connected to the chloroplasts and thus create a pool of chemically accessible hydrogen required for up-hill reactions in the path of carbon". It was assumed that the electrons flowing from cytochrome to chlorophyll are brought back again to cytochrome *b* by the transformed light energy. Arnon's discovery of photophosphorylation strongly supports this idea of the chloroplast cytochromes acting as a mechanism for transforming electrical energy into chemical energy.

The results of the present investigation are in agreement with Arnon's concept of a photophosphorylation and they also emphasize the similarities in enzymic composition of chloroplasts and mitochondria.

With regard to Arnon's distinction of a cyclic and a non-cyclic phosphorylation, or, in other words, production of only ATP or of ATP + TPNH, my own results point to the combined activity of a chain of reduction, ending in TPNH, and a chain of oxidation (through cytochromes) producing ATP. The following reactions may illustrate the main transferences (cf. Fig. 6):

$$\mathrm{TPN} + 2\mathrm{H}^+ + 2\downarrow e^- \xrightarrow[\text{chlorophyll}]{\text{excited}} \xrightarrow{\text{TPN reductase}} \mathrm{TPNH_2} \quad (1)$$

$$\mathrm{TPHN_2} + 2\mathrm{Fe}^{3+} \xrightarrow[\text{dehydrogenases}]{\text{co-factors}} \mathrm{TPN} + 2\mathrm{H}^+ + 2\mathrm{Fe}^{2+} \quad (2)$$

$$\mathrm{H_2O} + 2\mathrm{Fe}^{3+} \xrightarrow{\text{catalase ?}} \mathrm{O} + 2\mathrm{H}^+ + 2\mathrm{Fe}^{2+} \quad (3)$$

$$\mathrm{Fe}^{2+} + \text{excited chlorophyll} \rightarrow \text{chlorophyll} + \mathrm{Fe}^{3+} \quad (4)$$

Chloroplasts which are not performing chemosynthesis of carbohydrates only form ATP (Arnon's cyclic phosphorylation). The electrons which are relinquished from the excited chlorophyll are, together with protons from the surroundings, reducing TPN (reaction 1) and are from here (via dehydrogenase co-factors) transferred to cytochrome *b* (reaction 2). Because in reaction 2 the same quantity of protons is released as is consumed in reaction 1, hydroxyl ions are not produced. There is consequently no need for eliminating OH^- and producing oxygen. The electrons are flowing directly to the cytochromes from reduced pyridine nucleotide without accumulation of anything except ATP.

The need of a continuous supply of protons arises if $TPNH_2$ is used for an external purpose, such as the chemosynthesis of carbohydrates. Protons are, in this case, continuously pulled from water leaving OH^- ions which would be accumulating in unphysiological quantities unless they are eliminated by reac-

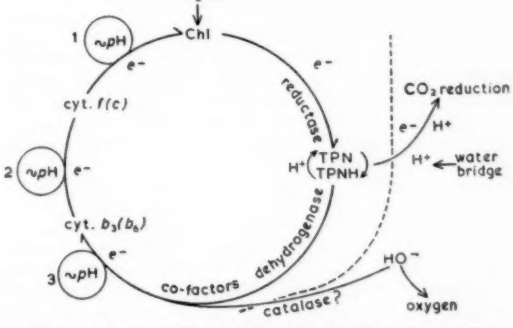

Fig. 6. Scheme of the photosynthesis in chloroplasts. The broken line marks the effect of switching carbohydrate synthesis on or off

tion 3. This process implies a de-ionization of OH^- by the hæm enzymes, possibly in co-operation with catalase, leaving reduced cytochrome *b* and oxygen. Reaction 3 is similar to the well-known Hill reaction[22], with the experimental difference that in the reaction observed by Hill, electrons from the excited chlorophyll are reducing external ferri-ions (cf. trapping of electrons in potassium ferrocyanide (Fig. 4)). What is producing oxygen is, however, in this case not the reducing moiety of the cycle but the oxidizing one, namely, the cytochrome system in co-operation with catalase or some other enzyme catalysing the electrolysis of water. If electrons are trapped in the reducing moiety the excited chlorophyll will pull electrons from the cytochromes and thus keep the electrolysis of water going. The relinquished protons (see reaction 3) are, in the Hill reaction, accumulating to unphysiological levels[23], but in normal photosynthesis they are feeding the transference of $H^+ + e^-$ from TPNH to the chemosynthetic processes producing carbohydrates from $CO_2 + H_2O$.

Introduction of water in the electron cycle means an extension of the electron transference to an outer circuit (the water bridge). The reducing moiety is here acting as the cathode and the oxidizing moiety as the anode. The electrons expelled from the excited chlorophyll are thus passing over the water bridge back again to the chlorophyll molecule (reaction 4).

The observed oxidation of the cytochromes on addition of TPN indicates a retardation or partial reversal of reaction 2. It may be expected that this initial retardation will decline slowly because of a simultaneous stimulation of reaction 1. The experiments show that the initial oxidation of the cytochromes soon disappears and even may turn over to reduction (Fig. 4).

On addition of TPNH, reaction 2 is going from left to right. This initial response, which results in an increased reduction of the cytochromes, soon declines after the speed of photophosphorylation has adjusted itself to the increased supply of electrons (Fig. 4).

The mere fact that the sudden start of the electronic cycle at turning on the light does not affect uniformly all links in the chain proves the existence of a spatial organization and of factors or zones of specific conductivity interspersed between the enzymes. Similar to the respiratory enzymes, the corresponding enzymes in chloroplasts are very probably organized in sub-micro-multimolecular groups[3] (layers, rod-like or helical formations, etc.) which are reacting at specific points ('reaction centres'[7]) or surfaces. A number of observations furthermore indicate the existence of intermediate factors[19], lipids or quinones, which are acting as electrical machines transforming the motive power of electrons into chemical energy. Most important are those machines which produce ATP. The intermediate factors are largely responsible for the internal conductivity of the system and the observed specific response of the various cytochromes may be interpreted as a reflexion of local changes in their conductivity.

The three points of phosphorylation are probably individually sensitive to variations of substrate concentrations, a circumstance that might explain negative results regarding the 'exchange reaction' between ADP and ATP [20]. It is important to distinguish between the initial response, which, according to the present article, mirrors the points of substrate sensitivity, and the adaptation of the total cycle to the changed conditions, which, as shown here, may be completed in less than 1 min. Conventional biochemical methods are insufficient for studying the initial effects. In yeast the mechanisms of oxidative phosphorylation were shown to be regulating the internal conductivity and hence the speed of electron transference from reduced substrates to oxygen. From the present results it may be inferred that in chloroplasts, too, the internal conductivity varies with the intensity of the ATP formation.

It has been shown that the external circuit (the water bridge in chloroplasts which are consuming both water and ATP during the chemosynthesis of carbohydrates) is sensitive to the presence of salts, in particular chloride, whereas salts are reported as not affecting the internal circuit[11]. This points to the existence of alternate pathways between TPN and the cytochromes as shown in Fig. 6. It appears from this scheme that the internal stream of electrons is automatically switched over to the water bridge the moment $H^+ + e^-$ are consumed for reduction of carbon dioxide.

In 1954 I advanced the idea that anions may, in chloroplasts, play a similar part as carriers of electrons as they are doing in the respiratory chain[5]. What is going on in the water bridge may be characterized as a hidden electrolysis of water (only oxygen is evolved, $H^+ + e^-$ is hidden in reduced products). Salts are promoting *in vitro* the electric conductivity and stimulating the electrolysis, and the same may be true regarding chloroplasts. The fact that chloride serves preferably as an electron carrier in photosynthesis may depend on the ability of chloride ions to be adsorbed firmly to the structure[21]. Also, the stimulating influence of salts on respiration may be specific, owing to hydration, specific mobilities, etc., of anions and cations, but the specificity is generally less pronounced than in the case of photosynthesis.

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[1] Lundegårdh, H., *Ark. Kemi* (Stockholm), **3**, 469 (1951); **5**, 97 (1952); **7**, 451 (1954).
[2] Lundegårdh, H., *Acta Chem. Scand.*, **10**, 1083 (1956).
[3] Lundegårdh, H., *Biochim. Biophys. Acta*, **27**, 653 (1958); **35**, 340 (1959).
[4] Lundegårdh, H., *Endeavour*, **16**, 193 (1959).
[5] Lundegårdh, H., *Physiol. Plant.*, **7**, 375 (1954).
[6] Hill, R., and Scarisbrick, R., *New Phyt.*, **50**, 98 (1951).
[7] Duysens, L. N. M., *Transfer of Excitation Energy in Photosynthesis* (Utrecht, 1952).
[8] Kok, B., *Nature*, **179**, 583 (1957).
[9] Hill, R., *Nature*, **174**, 501 (1954).
[10] Bendall, D. S., and Hill, R., *New Phyt.*, **55**, 208 (1956).
[11] Arnon, D. I., Allen, M. B., and Whatley, F. R., *Biochim. Biophys. Acta*, **20**, 449 (1956). Arnon, D. I., *Nature*, **184**, 10 (1959); *Cell-free Photosynthesis and the Energy Conversion Process.* (Symposium on Light and Life, Baltimore, 1960.)
[12] Vishniac, W., *Ann. Rev. Plant Physiol.*, **8**, 115 (1955).
[13] Granick, S., *Harvey Lectures, Ser.* 44, 220 (1950).
[14] Lundegårdh, H., *Nature*, **190**, 983 (1961).
[15] Bornstein, R., Goldberger, R., and Tisdale, H., *Biochem. Biophys. Res. Comm.*, **2**, Nr. 3 (1960).
[16] Warburg, O., *Heavy Metal Prosthetic Groups and Enzyme Action* (1949).
[17] Lewis, G. N., and Lipkin, D., *J. Amer. Chem. Soc.*, **64**, 2801 (1942).
[18] Witt, H. T., *Z. physik. Chemie*, N.F., **20**, 17, 283 (1959); **23**, 133 (1960).
[19] Lundegårdh, H., *The Cytochrome-Cytochrome Oxidase System. Encyclopedia of Plant Physiology*, Vol. 12, 311 (1960).
[20] Avron, M., *Biochim. Biophys. Acta*, **40**, 257 (1960).
[21] Lundegårdh, H., *Physiol. Plant.*, **11**, 342, 564, 585 (1958); **12**, 336, 342 (1959).
[22] Hill, R., *Nature*, **163**, 881 (1939).
[23] Clendenning, K. A., and Gorham, P. R., *Arch. Biochem. Biophys.*, **37**, 56 (1952).

LETTERS TO THE EDITORS

RADIOPHYSICS

Riometer Observations during a Solar Eclipse

DURING the solar eclipse of October 2, 1959, recordings of cosmic noise (extra-terrestrial radio waves) intensities were made with riometers at the Sagamore Hill Radio Observatory of the Air Force Cambridge Research Laboratories in Hamilton, Massachusetts. The observing site is 243 ft. above sea-level at 70° 48′ 56″ west longitude and 42° 37′ 51″ north latitude and was directly in the path of totality.

The riometer consists of a servo-controlled, self-balancing receiving system which compares the incoming cosmic noise signals with the noise output of a diode vacuum tube. Any change in the value of the input signal to the receiver is detected by an internal balancing system, and a corresponding change in the filament temperature of the noise diode is effected. The actual recorded trace is driven by the filament current of the servo noise diode and represents the power-level of the incoming cosmic noise at the antenna. Variations in the recorded signal strength from the normal day curve are due to fluctuations in the charged particle population of the atmosphere, in particular changes in the electron density of the ionosphere. The attenuation curve of a 27·5 Mc./sec. radio wave passing through the atmosphere is shown in Fig. 1 and was derived from electron density profiles and electron-neutral particle collision cross-sections (personal communications from T. J. Keneschea and G. Gassman).

On the day of the eclipse, the Sun rose approximately 10 min. before second contact. Totality began at 5 hr. 50 min. 1 sec. and continued until 5 hr. 51 min. 32 sec. E.S.T. The solar eclipse occurred at an elevation of about 1° above the horizon and was of magnitude 1·02. The Fort Belvoir and planetary *K* indices were 5 during the observing period.

Two riometers were used in the measurements. One was tuned to a frequency of 30·25 Mc./sec., with its antenna oriented toward the zenith. The other was tuned to 27·5 Mc./sec., and its antenna was pointed toward the eclipse. The antennæ were three-element Yagis with lobe dimensions of approximately 110° horizontally by 60° vertically. The 27·5 Mc./sec. riometer antenna was mounted a half-wave-length above the ground with its axis of maximum sensitivity about 30° above the horizontal. The azimuth position of this beam was 95° E., the azimuth angle at which the eclipse would take place. The beam-width of the 27·5 Mc./sec. antenna was sufficiently large to enable signals to be well received before and just after totality. Since the antenna was stationary and did not track the Sun, incoming signals were received in different parts of the antenna reception lobe as time progressed. The recorded fluctuations in signal strength, therefore, indicate only relative changes in absorption. Because of the large antenna beam-width, however, the 27·5 Mc./sec. record provided an excellent indication of the variation of atmospheric absorption as the eclipse progressed.

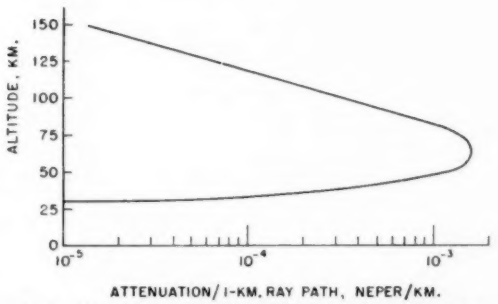

Fig. 1. Attenuation curve of a 27·5 Mc./sec. radio wave passing through the atmosphere

Fig. 2 shows the records of both the zenith and the eclipse riometers. The zenith riometer (30·25 Mc./sec.) shows no deviations from the normal quiet-day curve which was obtained from the records of the control days. The eclipse riometer record (27·5 Mc./sec.) shows slight absorption occurring before sunrise and an increase in cosmic noise of about 2 db. nearly coincident with optical totality. The pre-sunrise absorption may be due to the presence of auroral ionization observed in the backscatter records[1] of the Plum Island Observatory. It is of particular interest that peak cosmic noise intensity, corresponding to minimum electron density, is observed to follow optical totality by less than 30 sec.

As can be seen from Fig. 1, maximum attenuation for signals received by the eclipse riometer occurs at approximately 70 km., the *D* region. Because of the low angle at which the eclipse occurred, the path-length of the 27·5 Mc./sec. cosmic noise through the *D* region is about 200 km. Therefore, an average change of attenuation of 0·01 db./km. will explain the over-all change in attenuation of cosmic noise of

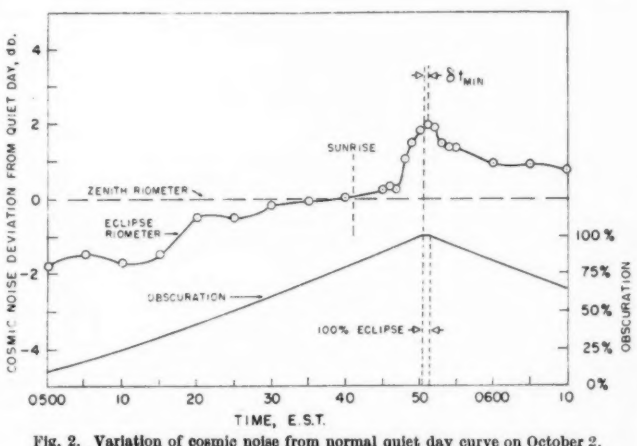

Fig. 2. Variation of cosmic noise from normal quiet day curve on October 2, 1959, during a solar eclipse. Percentage of obscuration also shown

2 db. At the zenith, the path-length is considerably less than the path-length in the direction of the eclipse. Therefore, the small change in attenuation observed in the eclipse riometer would not result in a noticeable change of cosmic noise intensity measured in the zenith.

Theoretical investigations of the E and F_1 regions during a solar eclipse have been made[2,3]. Expressions involving the delay in time between optical totality and the minimum electron density have been derived. Measured times of delay for the F_1 region during total eclipses are approximately 5 min.; for the E region, 3 min.[4]. From my measurement, the delay time, δ_{min}, is determined to be about 30 sec. for the D region. Such a value is consistent with theory.

This work has been sponsored by a National Science Foundation grant to the Arctic Institute of North America. It was carried out at the Ionospheric Physics Laboratory, United States Air Force Cambridge Research Laboratories, Massachusetts. I thank H. Strick, of the Radio Astronomy Branch, for operation of the Zenith riometer and analysis of the zenith observations.

M. A. Gordon

Arctic Institute of North America,
1530 P Street, N.W., Washington 5, D.C.

[1] Malik, C., Aarons, J., and Poeverlein, H., *J. Geophys. Res.*, **65**, 3241 (1960).
[2] Rydbeck, O. E. H., and Wilhelmsson, H., *Avd. Elektroteknik*, **41**, No. 149, 14 (1954).
[3] Bates, D. R., and McDowell, M. R. C., *J. Atmos. Terr. Phys.*, **10**, 96 (1947).
[4] Ratcliffe, J. A., *Solar Eclipses and the Ionosphere*, 1 (Pergamon Press, 1956).

GEOPHYSICS

World-wide Distribution of Diurnal Variation of the Neutron Intensity of Cosmic Rays

It is important to glean information on ways in which cosmic rays are influenced in interplanetary space. If it were possible to find world-wide distributions of cosmic rays and their variations with time, one might be able to obtain information on modulation of cosmic rays by magnetic clouds in interplanetary space. As it is impossible, however, to normalize the cosmic-ray intensities at every station in the world at present, I investigated the world-wide distributions of local times of maximum intensity in the diurnal variations and their variations with time during the period of the International Geophysical Year, and the following results were obtained:

Data of the thirty-nine stations in the world were used during the period July–December 1957; forty-five stations during January–June 1958; and forty-five stations during July–December 1958. These stations were classified into two groups; one is the mountain altitude group ($\geq$ 2,000 m. above sea-level), the other the sea-level group ($<$ 2,000 m. above sea-level).

T_m, the local time of the maximum intensity in the diurnal variation, was derived as follows:

(1) The bi-hourly readings, represented by the procedure of Wada[1], were averaged over a period of one month, excluding all data of the imperfect days, and only the months having more than ten perfect days were used. The harmonic analyses were carried out on these monthly mean diurnal variations, and the average value of T_m of each month was derived from the vector sum of the first and second harmonics.

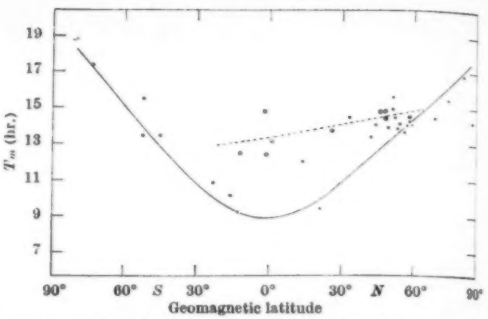

Fig. 1. Relation between T_m and the geomagnetic latitude on the average, during the period July–December 1957. Full line and ●, sea-level group; dotted line and ○, mountain altitude group

(2) The average values of T_m during the period of each half-year were derived from the averaged values of the vector sum during six months of each period.

Variations of T_m with the geomagnetic latitudes during the period of July–December, 1957, are shown in Fig. 1. It seems that there is a latitude effect in T_m, which has the minimum value around the geomagnetic equator. Also the minimum values of T_m and the differences of T_m ($\Delta T_{m,\ 80°-0°}$) between 80° and 0° (geomagnetic latitude) of the latitude-effect curves belonging to the sea-level group have both clear correlations with the sunspot numbers determined by the Zurich Astronomical Observatory during each month. It appears that minimum T_m advances and $\Delta T_{m,80°-0°}$ increases as the solar activity becomes intense. Since many points deviate from the curve in Fig. 1, lines of equal T_m were drawn in the world map in order to find reasons for this. From the lines of equal T_m in the world map (Mercator's projection), it is seen that world-wide distributions have special tendencies: there is a minimum value of T_m in the region from the Pacific Ocean to South Africa passing through South America, there are longitudinal effects, and the distributions tend to move every month, as shown in Fig. 2.

If it is possible to define broadly the minimum point between the northern hemisphere and the southern hemisphere as producing the equator, it is then possible to consider the minimum point of T_m as a kind of cosmic-ray equator, instead of the minimum point of the intensity, and this may be important, since one cannot normalize the intensity of stations. Thus it may be plausible to suggest that the cosmic-ray equator is moving with time.

From the results mentioned before, it may be suggested that the cosmic-ray intensity is controlled by three factors: the first is due to the inner terrestrial magnetic dipole, the second due to the modulation by magnetic clouds in interplanetary space, and the third due to deformation of the lines of force of the geomagnetic field by contact with magnetic clouds.

It may be suggested that, from the first factor, there exists the latitude effect on the average; from the second, minimum T_m advances and $\Delta T_{m,\ 80°-0°}$ becomes large as the solar activity becomes intense; and from the third are derived variations with time of the world-wide distribution of T_m and the cosmic-ray equator.

It was pointed out by Simpson *et al.*[2] and others[3] that a difference between the geomagnetic equator and

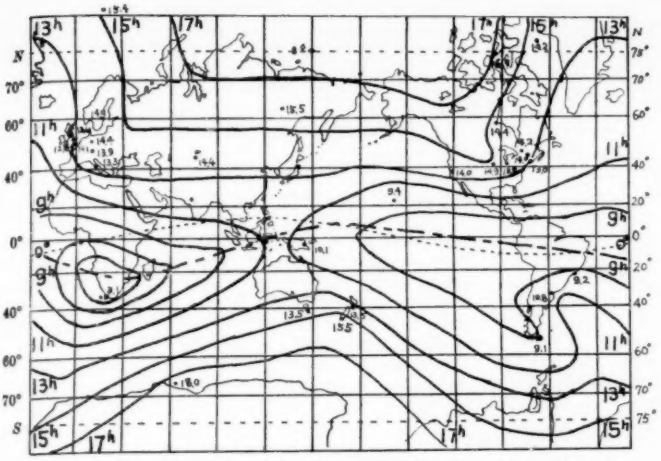

Fig. 2. The average world-wide distribution of T_m during the period July–December 1957. The heavy chained line shows the cosmic-ray equator determined by T_m. The fine dotted line shows the geomagnetic equator. Numbers at stations show T_m (units/hr.) belonging to them

the cosmic-ray equator exists, but the reason for this has not been made clear until now. The cosmic-ray equator, that was established by them, was produced from data obtained at different times. If it is true that the location of the cosmic-ray equator may also vary with time because the cosmic ray is modulated in interplanetary space, it may be permissible to determine the location of the cosmic-ray equator without consideration of the variation with time.

I thank the directors and staffs of all the stations from which data were obtained for this work. These data were obtained through *WDC*–C2 by Dr. Y. Miyazaki *et al.*, whom I wish to thank too. Also my thanks are due to Prof. Y. Kato for his suggestions and continuous encouragement, to Prof. Y. Sekido, the chief director of the Primary Cosmic-Ray Co-operative Research Group in Japan, and members of this group for their help.

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TSUNEKICHI KANNO

Department of Physics,
Fukushima University,
Fukushima, Japan.

[1] *Cosmic-Ray Intensity during the International Geophysical Year* No. 1, No. 2 and No. 3 (Science Council of Japan, Tokyo).

[2] Simpson, J. A., Fenton, K. B., Katzman, J., and Rose, D. C., *Phys. Rev.*, **102**, 1648 (1956).

[3] Oguchi, T., and Kodama, M., *Nature*, **183**, 103 (1959). Kodama, M., *J. Geomag. Geoelect*, (Kyoto), **10**, 37 (1959).

Effect of a Lake on Distribution of Permafrost in the Mackenzie River Delta

THE thawing effect of water in contact with permafrost is of considerable importance to engineers engaged on construction work that may involve flooding areas of perennially frozen ground. There is little information at present available, however, to indicate the magnitude of the effect which might be expected. An investigation of the present level of permafrost under natural bodies of water in the north, such as lakes and streams, is one means of improving our knowledge of this effect and of providing some guidance to engineers. During April 1961 a drilling programme was carried out by the Division of Building Research of the Canadian National Research Council to determine the distribution of permafrost under a lake in the Mackenzie River Delta (68° 18′ N., 133° 50′ W.) at the new town-site of Inuvik, Northwest Territories, near the arctic coast.

A small lake was selected for investigation following a preliminary study of aerial photographs and a field reconnaissance, in 1960, of a portion of the delta within a 10-mile radius of Inuvik. This lake, located about 5 miles south-west of Inuvik, is approximately circular, having a diameter of about 900 ft. Like all delta lakes it is shallow. At the time of investigation the ice cover was about 2·25 ft. thick and the average depth of water was 1·5–2 ft. The snow cover was on the average 12–15 in. The colour of the lake water was light brown and a very strong odour of hydrogen sulphide was noticed when the bottom sediments were disturbed. The dense forest vegetation around the lake is typical of the climax developed in the lower valley and delta of the Mackenzie River. The dominant species is white spruce, the largest trees growing to a height of 50 ft. Also present are black spruce, willow and alder with scattered stands of tamarack. The apparently continuous moss cover is about 1 in. thick.

A diamond drill-bit was used to drill and sample the various materials encountered. The operation was greatly complicated by the presence of permafrost and the subnormal air temperatures which occurred during April. Water, obtained from the lake at a temperature of 33°–35° F., was used as a circulating medium except for an unsuccessful attempt to use arctic grade fuel oil in one hole. When using water, drilling techniques had to be slightly modified and precautions taken to prevent freezing of equipment in the hole. Hot water was circulated periodically to keep the hole free of ice.

During the course of the investigation, four holes were drilled at the locations and to the depths noted: No. 1, centre of lake—to 240 ft.; No. 2, 33 ft. west of lake—to 115 ft.; No. 3, 132 ft. west of lake—to 196 ft.; No. 4, 550 ft. west of lake—to 260 ft.

To measure ground temperatures, two thermocouple cables were installed to depths of 100 and 200 ft. in holes Nos. 3 and 4 respectively.

Good undisturbed cores were obtained which indicated the following soil profile: 0–100 ft., thinly stratified sandy silt with layers of decomposed organic material throughout; 100–180 ft., fine to medium sand with thin layers of organic material in the upper 40 ft.; 180–240 ft., very dense, clay-sized soil (free of stones except for last few feet).

Representative samples were taken for identification and classification testing of the soils, moisture (ice) content, pollen analysis, carbon-14 age determinations and petroleum pigment analysis. Bedrock (a dolomitic limestone) was encountered in hole No. 3 at a depth of 230 ft. and in hole No. 4 at a depth of 260 ft.

Although testing of samples has not been completed nor the information fully analysed, a number of observations can be made. No frozen ground was encountered throughout the depth of the hole under the lake (No. 1), but did occur for the full depth of each of the three other holes. Initial ground temperature measurements suggest that the permafrost is about 250 ft. thick at the location 130 ft. from the lake and about 300 ft. thick at a distance of 550 ft. from the lake.

Probings showed that the permafrost table extended out from shore under the lake and sloped down steeply. 10 ft. from the shore the permafrost table occurred 15·5 ft. below the ice surface; 12 ft. from the shore at a depth of 19·7 ft.; at 15 ft. it was more than 22 ft. below the ice. Although the soils encountered in the three holes adjacent to the lake (Nos. 2, 3 and 4) were solidly frozen, little ice was observed in the soils below the 20-ft. depth. Although flooding of the lake and surrounding area probably does not occur each year, it appears that it may occur at intervals of a few years. Marks on tree trunks indicate an extreme high-water mark about 5 ft. above the present ground surface at the lake, that is, about 20 ft. above the winter ice-level of a nearby delta channel.

G. H. Johnston
R. J. E. Brown

Northern Research Group,
Soil Mechanics Section,
Division of Building Research,
National Research Council, Ottawa.

METEOROLOGY

Size Distribution of Raindrops

A. C. Best[1] has found an empirical formula for the size distribution of raindrops. If $F(x)$ is the fraction of liquid water in the air comprised by drops of diameter less than x:

$$F = 1 - \exp[-(x/a)^n]$$

where a and n are constants for any particular rainfall. Contrary to the general supposition that the above formula applies to the average size distribution of a number of samples of rain, I have found that the formula holds for individual samples in 90 out of 104 cases of general monsoon rains and in 134 out of 169 cases of thunderstorm rains recorded at Poona, provided the samples are sufficiently representative. The formula fails where the liquid water distribution is multimodal (generally bimodal) or where the distribution curve has a long tail with a sharp maximum either at the beginning or at the end.

The parameters a and n are found to be independent for general rains but are connected by the average relation $an = 7{\cdot}1$ mm. for thunderstorm rains. There is also a correlation between the intensity of precipitation I (mm./hr.) and the parameter n. For general rains the average relation found is:

$$I = 6{\cdot}9 \exp[-0{\cdot}14(n - 5{\cdot}5)^2]$$

and that for thunderstorm rains is $I = 975 \exp(-n)$.

V. N. Kelkar

Nowrosjee Wadia College,
Poona, India.

[1] Best, A. C., *Quart. J. Roy. Met. Soc.*, **76**, 16 (1950); **77**, 418 (1951).

PHYSICS

Stark Broadening of the Spectrum Line 4603 Å. of Lithium

The Stark broadening of spectrum lines, in particular the lines of hydrogen, has been of interest to astrophysicists for many years. Recent advances in plasma physics on a laboratory scale have emphasized the need for a better understanding of the relationship between the number density of charged particles in a plasma and the profiles of the spectrum lines from the plasma. The broadened hydrogen lines from a water-stabilized carbon arc have been studied carefully by workers at Kiel[1-3], and the latest refinements of the theory of line broadening describe the profile of Hβ quite accurately[4].

The shock tube is a very useful device for studying spectrum lines from plasmas, since the degree of ionization and the temperature behind the shock wave can be varied over a wide range and calculated from measurements of the shock speed. The shock tube used for the present work has a low-pressure section 18 ft. 7 in. long, and a high-pressure section 3 ft. long. Argon is used as the test gas in the low-pressure channel, and hydrogen as the driver gas in the high-pressure section. Diaphragms of aluminium or 'Mylar' are used, depending on the bursting pressure desired. Spectra are obtained with a Raman glass spectrograph. Spectra can be resolved in time by sweeping the spectrograph slit with a rotating disk having a number of radial slots.

Lithium was chosen for initial Stark broadening studies, since this shows a large Stark effect for some of its lines, particularly the line at 4603 Å. Hydrogen and helium both have large Stark shifts, but were not used since their presence in quantities large enough to give easily photographed spectrum lines would reduce the shock strength quite markedly. The lithium was introduced into the shock tube in the form of lithium chloride which was supported on a short rod mounted on the closed end of the tube. A time-resolved spectrum showed that the lithium was excited behind the shock wave reflected from the closed end of the tube. A time-integrated spectrum is shown in Fig. 1. The argon lines are broader than those emitted from an argon discharge lamp and they are also shifted a little to the red. Hβ appears very faintly; this is excited near the contact surface between the hydrogen driver and the argon. The lithium line at 4603 Å. is very broad. The blackening profile of this line is shown in Fig. 2.

In the analysis of this line it has been assumed that the line is broadened solely by the statistical microfields due to ions. The broadening of the lithium line at 4603 Å. has been treated approximately by Holstmark using some experimental results obtained by Stark on sparks through lithium vapour[5]. Since then, more accurate Stark results have become available for lithium mainly through the experimental work of Poirot and these results have been used for the present analysis[6].

The lithium line at 4603 Å. arises from a $2p$–$4d$ transition. Under the action of a uniform electric field this line is displaced to longer wave-lengths and two other lines appear, one due to a $2p$–$4p$ transition and the other to a $2p$–$4f$ transition. The $2p$–$4p$ transition gives a weak line at 4636 Å. for vanishingly weak electric fields, shifting to longer wave-lengths at increasing field strengths.

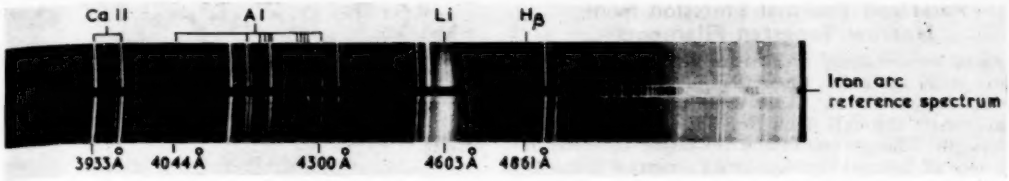

Fig. 1. Lithium excited in the shock tube, showing Stark-broadened line at 4603 Å. The Stark-broadened hydrogen line Hβ is just visible also

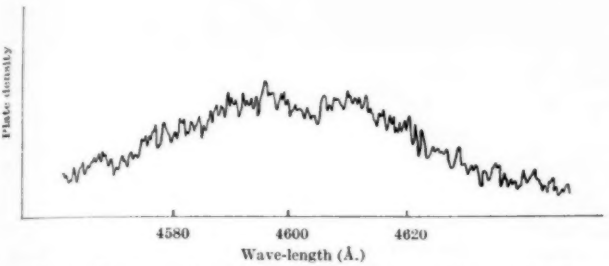

Fig. 2. Density profile of Stark-broadened line of lithium at 4603 Å.

The $2p$–$4f$ transition gives a line at 4602 Å. for vanishingly weak fields, shifting to shorter wave-lengths at increasing field strengths. Data on the intensities of these Stark components are not available and therefore the full-line contour cannot be worked out as it has been done for the hydrogen lines by Schmaljohann, who applied Holtsmark's statistical theory to the known Stark data for hydrogen[7]. In order to overcome this difficulty it is assumed that the long wave-length part of the contour, having a maximum at 4611 Å., is due solely to the $2p$–$4d$ transition, and the weaker $2p$–$4p$ transition, which is much farther into the red, is neglected. This part of the line contour cannot be encroached on by the line due to the $2p$–$4f$ transition, since that cannot affect any part of the line at wave-lengths greater than 4602 Å. Poirot gives only rough estimates of the intensities of the components, but from these estimates it appears that the line due to $2p$–$4d$ transition does not vary in intensity over the field strengths he used, which did not exceed 95 kV. per cm.

Poirot's results show that the Stark displacement varies very nearly as the square of the electric field strength. For the quadratic Stark effect, the statistical theory of line broadening for a single line leads to a line profile given by (ref. 8, equation 3·14):

$$I = \frac{W(\beta)}{2\nu_0\beta} \qquad (1)$$

where

$$\beta = \left(\frac{\Delta\nu}{\Delta\nu_0}\right)^{1/2} \qquad (2)$$

where $\Delta\nu$ is Stark displacement in cm.$^{-1}$ due to a field F, $\Delta\nu_0$ is Stark displacement in cm.$^{-1}$ due to a field F_0 and W is a probability function that has been tabulated by Schmaljohann[7].

F_0 is related to the number density of ions, N, by:

$$F_0 = 2{\cdot}61\ eN^{2/3} \qquad (3)$$

where e is the electronic charge ($4{\cdot}80 \times 10^{-10}$ E.S.U.).

From Schmaljohann's tabulated values of $W(\beta)$, it is readily found that the maximum of I occurs when $\beta = 1{\cdot}1$. Substitution in (2) for β gives the position of the maximum as $1{\cdot}21\ \Delta\nu_0$. From direct measurement of the blackening contour (Fig. 2), this is 38 cm.$^{-1}$, which gives $\Delta\nu = 31{\cdot}4$ cm.$^{-1}$. From an extrapolation of Poirot's results this corresponds to $F_0 = 122$ kV./cm.

Equation (3) then gives:

$$N = 1{\cdot}85 \times 10^{17} \text{ per c.c.}$$

In the actual experiment the shock wave Mach number was 9·2, and the initial pressure of the argon 5 mm. mercury. By calculation this gives a temperature of 11,500° K. and ion density of $1{\cdot}6 \times 10^{17}$ per c.c. behind the shock wave reflected from the end of the tube. There are two effects which would lead to a higher observed ion density than that computed behind the reflected shock wave: (1) the reflected shock wave undergoes a second reflexion at the contact surface before the radiation is finally quenched by the spreading contact surface. This second reflexion is shown by a time-resolved spectrum, and has the effect of further heating and compressing the gas, thus raising the number density of electrons and ions. Computations have not been carried out for this second reflexion. (2) The lithium itself almost certainly provides a large number of electrons and ions since this has a much lower ionization potential (5·39 eV.) than argon (15·75 eV.).

This work is proceeding further in order to investigate the effect of varying the number density of ions and electrons. The most recent work on hydrogen line broadening also shows the need to take into account the effect of electrons on the broadening, which has been neglected in this treatment[9].

This work is published by permission of the Director of the Laboratory. I acknowledge with thanks the assistance of Mr. J. Townsend in the experimental work.

K. C. Lapworth

Aerodynamics Division,
National Physical Laboratory,
Teddington,
Middlesex.

[1] Jürgens, G., *Z. Phys.*, **134**, 21 (1952).
[2] Griem, H., *Z. Phys.*, **137**, 280 (1954).
[3] Bogen, P., *Z. Phys.*, **144**, 62 (1957).
[4] Griem, H., Kolb, A. C., and Shen, K. Y., *Phys. Rev.*, **116**, 4 (1959).
[5] Holtsmark, J., *Ann. der Phys.*, **58**, 577 (1919).
[6] Poirot, A., *Annales de Phys.*, **4**, 533 (1935).
[7] Schmaljohann, P., *Staatsexamens-Arbeit, Kiel* (1936).
[8] Margenau, H., and Lewis, M., *Rev. Modern Phys.*, **31**, 569 (1959).

Polarized Thermal Emission from Narrow Tungsten Filaments

From experimental work carried out by Fresnel many years ago and a theoretical investigation made by K. Schwarzschild[1], it is well known that light transmitted through a narrow slit is more or less polarized. Though conditions are rather different in the case of thermal emission from a narrow filament the idea has occurred to me that polarized emission may be obtained in this way in addition to inclined-surface-layer-effects described by Worthing[2] and others (Arago effect[3]). Through the courtesy of Dr. G. Siljeholm of the Lumalampan Aktiebolag in Stockholm I have been able to test recently some filaments no thicker than 4·8μ. The experiments were carried out at the Swedish Solar Observatory in Anacapri, and a polarization as high as 28 per cent has been found in the red. The plane of vibration (electric vector) is perpendicular to the direction of the filament. Considering the fact that the 4·8μ filament had not been polished as in the case of Worthing's measurements, the high value obtained in the red (28 per cent instead of 19 per cent found by Worthing) gives some indication that a 'slot-aerial' effect might be present. To some extent this view is supported by measurements of a 6·5μ filament showing already a smaller degree of polarization than a polished broad filament. Furthermore, when examining with a Savart polariscope the interference fringes appearing in highly magnified images, it has been found that the polarization appears to be nearly the same in the central part of the wire as at the limb. Though the resolving power may have been insufficient for separating the regions clearly, it seems hard to explain the observation by means of the Arago effect alone.

By using different colour screens I have been able to establish an increase of polarization with wave-length. The determinations are presented in Table 1. The effective wave-length λ transmitted through the filters has been estimated with a spectroscope. The degree of polarization was measured with a Cornu polarimeter, except in the case of infra-red radiation for which photographic observations were made. Special precautions were taken to avoid polarization effects due to reflexion in the glass tube, etc.

In Table 1 the quantity $\frac{d}{\lambda}$ is given as well as $P\frac{d}{\lambda}$, d being the thickness of the wire. As is evident from the last column $P\frac{d}{\lambda}$ is remarkably constant and approximates to 2.

In Fig. 1 the degree of polarization is plotted against $\frac{d}{\lambda}$ using dots for $d = 6{\cdot}5\mu$ and crosses for $d = 4{\cdot}8\mu$. The dots follow closely a straight line. All values follow closely the hyperbola $P\frac{d}{\lambda} = 2$ presented as a dashed curve. When using instead $1/\lambda$ as abscissa the crosses and the dots give different relationships. This indicates again that an aerial effect may be present. The

Table 1

$d(\mu)$	$\lambda(\mu)$	$\frac{d}{\lambda}$	P	$P\frac{d}{\lambda}$
6·5	0·48	13·5	0·14 ± 0·02	1·9
	0·55	11·8	0·16 ± 0·01	1·9
	0·64	10·2	0·16 ± 0·02	1·6
	0·66	9·8	0·19 ± 0·02	1·9
	0·82	7·9	0·22 ± 0·02	1·7
4·8	0·48	10·0	0·22 ± 0·02	2·2
	0·66	7·2	0·28 ± 0·02	2·0

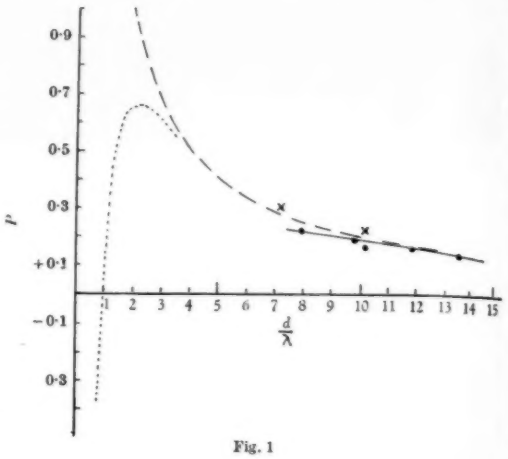

Fig. 1

probable behaviour close to $\frac{d}{\lambda} = 1$ has been indicated by the dotted curve by taking into account the phenomena produced by a narrow slit. If the relation $P\frac{d}{\lambda} = 2$ is valid also for smaller values of $\frac{d}{\lambda}$ than those examined here, the highest polarization would be expected when the thickness of the filament is about twice the wave-length of light.

Preparations are being made for extending the measurements to narrower filaments. It is planned to ascertain also whether narrow bolometer filaments may be used for the purpose of analysing polarization in the infra-red.

Yngve Öhman

Stazione Astrofisica Svedese,
Anacapri,
Italy.

[1] Schwarzschild, K., *Math. Ann.*, **55**, 177 (1901). Compare also similar effects produced in gratings (Riecke, *Lehrbuch der Physik*, **2**, 375; 1919).

[2] Worthing, A. G., *J. Opt. Soc. Amer.*, **13**, 635 (1926).

[3] Öhman, Y., *Polarization Measurements in Astronomy* (Boulder, 1949).

CRYSTALLOGRAPHY

Crystallographic Data for Urea Calcium Nitrate and Urea Cadmium Nitrate

The crystal structures of urea calcium nitrate and urea cadmium nitrate are being investigated as part of a survey of hydrogen bonding in urea inorganic salt complexes. Preliminary work has shown that urea calcium nitrate and urea cadmium nitrate are isostructural and belong to the monoclinic system.

By using small single crystals mounted on a Seifert diffractometer and copper K_α radiation, accurate values for the translations d_{001}, d_{100}, d_{101} and b were obtained. The accurate estimates were made by plotting the values of these translations, determined from the $00l$, $h00$, $h0l$ (for $h = 1$) and $0k0$ reflexions, against the function[1] $\frac{1}{2}\left(\frac{\cos^2\theta}{\sin\theta} + \frac{\cos^2\theta}{\theta}\right)$ and extrapolating to zero.

The monoclinic angle β was calculated by means of the equation:

$$\beta = \arccos \frac{d^2_{101}(d^2_{100} + d^2_{001}) - (d_{100}d_{001})^2}{2d_{100}d_{001}d^2_{101}}$$

By using the calculated value of β and the observed values of d_{001} and d_{100}, the unit cell translations c and a were derived.

The systematic absences were deduced from indexed Weissenberg photographs taken about the unit cell axes.

The densities were determined by flotation of the crystals in toluene/bromoform mixtures.

A summary of the crystallographic data for urea calcium nitrate and urea cadmium nitrate is given in Table 1.

Table 1

	Urea calcium nitrate, $Ca(NO_3)_2{\cdot}4(NH_2)_2CO$	Urea cadmium nitrate, $Cd(NO_3)_2{\cdot}4(NH_2)_2CO$
a	7·683 ± 0·002 Å.	7.607 ± 0·002 Å.
b	12·665 ± 0·002 Å.	12·772 ± 0·002 Å.
c	9·245 ± 0·002 Å.	9·040 ± 0·002 Å.
β	113° 32′ ± 2′	114° 4′ ± 2′
No. of molecules per unit cell	2	2
Measured density	1·634 gm. cm.$^{-3}$	1·968 gm. cm.$^{-3}$
Calculated density	1·628 gm. cm.$^{-3}$	1·973 gm. cm.$^{-3}$
Linear absorption coefficient for copper $K\alpha$ radiation	41·9 cm.$^{-1}$	123·0 cm.$^{-1}$
Systematic absences		
$h0l$	$h = 2n + 1$	$h = 2n + 1$
$0k0$	$k = 2n + 1$	$k = 2n + 1$
Space group (uniquely determined)	$P2_1/a$	$P2_1/a$
$a/2 - c\cos\beta$	0·150	0·118

All the crystals of urea cadmium nitrate that were examined were found to be twinned, with (010) forming the composition plane. Examination of the (010) Weissenberg photographs of urea cadmium nitrate showed that twinning had occurred as a result of a two-fold rotation about the a axis. Since the relation $c\cos\beta = \frac{a}{2}$ holds fairly closely for urea cadmium nitrate, this is an example of twinning according to the Carlsbad Law[2].

No evidence of twinning has been observed in the isostructural urea calcium nitrate complex, for which the value of $\frac{a}{2} - c\cos\beta$ is equal to 0·150 as compared with the value of 0·118 for urea cadmium nitrate.

C. G. C. Catesby

Research Department,
African Explosives and Chemical Industries, Ltd.,
P.O. Northrand,
Transvaal, South Africa.

[1] Henry, N. F. M., Lipson, H., and Wooster, W. A., *The Interpretation of X-Ray Diffraction Photographs*, 190 (Macmillan, London, 1953).
[2] Bragg, W. L., *Atomic Structure of Minerals*, 244 (Cornell Univ. Press, New York, 1937).

CHEMISTRY

A Transition in the Dielectric Properties of Solid Secondary Alcohols

Since the discovery of the large dielectric absorption in aliphatic secondary alcohols[1], there have been several papers[2–4] published on this subject. The only definite conclusion about the mechanism is that the presence of some lattice defect or arrangement of defects is required for this large absorption[3,4].

In the belief that further progress can be made only from measurements on single crystals grown from pure material, attempts have been made to grow single crystals from the melt. The solid grown to date has been below the standard it is hoped to obtain eventually. Nevertheless, measurements made on the oriented polycrystalline material obtained has revealed a sharp transition in the dielectric properties of certain alcohols at a temperature about − 4° C. The transition was not apparent in samples pressed from powders, although a suggestion of its presence was noted in samples annealed for long periods.

The solid was that used by Meakins and Sack[1], zone-refined and grown from the melt by a Bridgman method. The secondary alcohol was contained in a glass tube which was evacuated to a pressure of 10^{-5} mm. mercury. The tube was lowered through a sharp and highly stable temperature gradient at 0·5 in. a day. After removal from the tube, plane parallel sections for measurement were cut in three perpendicular directions, using a wet string saw. One of these directions was perpendicular to the growth axis. In some experiments 'Aquadag' electrodes were painted on the samples.

In the case of 9-heptadecanol ($C_8H_{17}CHOH.C_8H_{17}$), the absorption at room temperature is comparable in all three directions. However, the change in dielectric absorption at the transition is three times greater in the two directions for which the measuring field is perpendicular to the growth axis than when the field is in the direction of the growth axis. In Fig. 1 the dielectric absorption for one of these samples is

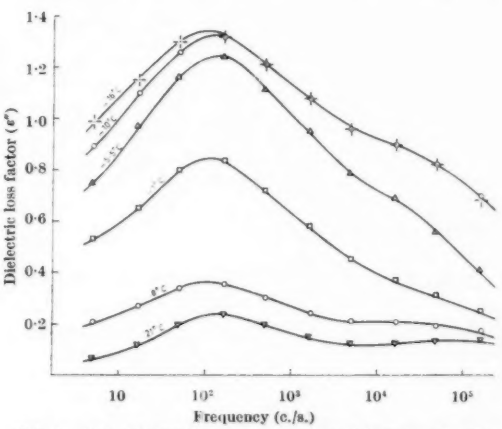

Fig. 1. Dielectric-loss factor as a function of frequency for the symmetrical secondary alcohol 9-heptadecanol

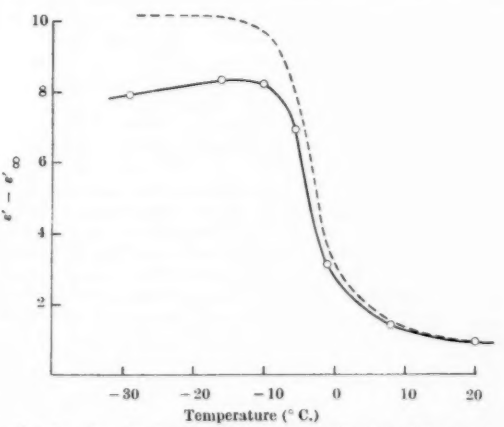

Fig. 2. $\varepsilon' - \varepsilon'_\infty$ for 9-heptadecanol as a function of temperature. The full line refers to ε' measured at 4·8 c./s., the dotted line to an estimated ε'_s, that is, the permittivity below the absorption region

plotted as a function of frequency at several temperatures. In Fig. 2 the relative permittivity at a frequency of 4·8 c./s. is plotted as a function of temperature; the dotted curve is an estimated ε'_0 obtained by extrapolating the absorption curves to frequencies below 4·8 c./s. and measuring the area under the extrapolated portion. It can be seen that the dispersion ($\varepsilon'_0 - \varepsilon'_\infty$) increases by a factor of 10(0·95–10) in passing through the transition.

A similar transition at approximately the same temperature has been observed in 8-pentadecanol ($C_7H_{15}CHOH.C_7H_{15}$), and evidence has been obtained of a less-sharp transition in 10-nonadecanol ($C_9H_{19}CHOH.C_9H_{19}$). The best sample obtained of the latter material was considerably more polycrystalline than those of the other compounds.

A direct-current voltage of 30 kV./cm. was applied to the samples at temperatures both above and below the transition; the permittivity was linear up to this voltage.

Further purification and growth of better crystals of these compounds are being attempted.

We wish to acknowledge the contributions of Mr. J. S. Cook and Mr. K. G. Weir to the crystal-growing apparatus.

J. S. Dryden
H. K. Welsh

Division of Electrotechnology,
National Standards Laboratory,
Commonwealth Scientific and Industrial
Research Organization,
Chippendale, New South Wales, Australia.

[1] Meakins, R. J., and Sack, R. A., *Austral. J. Sci. Res.*, **4**, 213 (1951).
[2] Dryden, J. S., and Meakins, R. J., *Rev. Pure and App. Chem.*, **7**, 15 (1957).
[3] Welsh, H. K., *Trans. Farad. Soc.*, **55**, 235 (1959).
[4] Daniel, V., *Trans. Farad. Soc.*, **54**, 1834 (1958).

1,2-Benzanthracene Derivatives in a Kuwait Mineral Oil

The isolation of a number of polymethylanthracenes from fractions of a Kuwait crude oil has been reported by one of us[1]. The hydrocarbons were obtained by treatment of acetone extracts of the oil with maleic anhydride and chromatography of the material recovered from the acidic adducts by distillation from sodium hydroxide. We have now applied the same procedure to higher-boiling fractions of acetone and furfural extracts of the Kuwait oil (corrected boiling points in the range 390–440° C.) and have obtained crystalline fractions consisting essentially of alkyl derivatives of 1,2-benzanthracene. This is evident from their ultra-violet absorption spectra, which closely resemble those of typical alkylbenzanthracenes[2]. Vapour-phase chromatography of some of the fractions indicates, however, that they are mixtures containing varying proportions of benzanthracene and other components with retention times similar to those of mono- and dimethylbenzanthracenes. 1,2-Benzanthracene itself was isolated from several fractions by further chromatography on alumina as colourless plates, m.p. 154–156° undepressed, when mixed with an authentic specimen, m.p. 158–159°. The ultra-violet spectrum of the isolated material ($\lambda_{max.}$ 228, 256, 267, 277, 287; 299, 314, 327, 342, 358; 374, 384 mμ; log ε, 4·54, 4·51, 4·64, 4·89, 4·98; 3·95, 3·70, 3·80, 3·85, 3·69; 2·69, 2·86), and the infra-red spectrum[3], were identical with those of 1,2-benzanthracene, and vapour-phase chromatography gave one peak with retention time similar to that of benzanthracene under the same conditions. The 2,4,7-trinitrofluorenone addition complex formed red needles, m.p. 217–221°, not depressed when mixed with an authentic specimen, m.p. 220–223° [4], and the identification was confirmed by preparation of the *s*-trinitrobenzene complex, orange needles, m.p. 161–163°, and comparison of this with an authentic specimen, m.p. 162–163° (found: C, 65·3; H, 3·6; $C_{24}H_{15}N_3O_6$ requires C, 65·3; H, 3·4 per cent).

A second product, m.p. 192–194·5°, obtained in very small amount after purification by chromatography and sublimation, did not depress the melting point of 4′-methyl-1,2-benzanthracene (m.p. 199–200°)[5], and the ultra-violet spectrum ($\lambda_{max.}$ 226, 228–9, 256, 271·5, 281·5, 292·5; 302, 316, 328·5, 344, 361; 387 mμ; log ε, 4·55, 4·55, 4·57, 4·65, 4·78, 4·79; 4·02, 3·77, 3·80, 3·81, 3·66, 2·94) was identical with that of this hydrocarbon. The infra-red spectra were also identical in the region 650–900 cm.$^{-1}$ (there was insufficient material to obtain a well-defined spectrum over the remainder of the normal range)[3]. The 2,4,7-trinitrofluorenone complex formed brown needles, m.p. 223–227°, and when mixed with the derivative of 4′-methyl-1,2-benzanthracene (m.p. 233–235°)[6] the melting point was 224–230°. The *s*-trinitrobenzene complex gave red needles, m.p. 158–160°; mixed with the derivative of the 4′-methyl compound, m.p. 165·5–168°, the melting point was 160–168°. Because of the very small amounts of material available it was not possible to obtain the derivatives completely pure, but the results reported, taken as a whole, may be considered to justify the identification of the substance from the oil as 4′-methyl-1,2-benzanthracene.

The presence of derivatives of 1,2-benzanthracene in fractions of the Kuwait oil is of interest with regard to the carcinogenic activity of the oil[7]. We have not as yet been able to obtain an accurate estimate of the amount of benzanthracenes present; but spectroscopic evidence indicates that, in one fraction of corrected boiling point 390–405°, it is certainly less than 0·25 per cent of the acetone extract, disregarding possible losses during decomposition of the maleic anhydride adducts. 1,2-Benzanthracene itself is generally considered to be non-carcinogenic or only very weakly so when tested by skin painting on animals[8,9], but it has been reported to be moderately active by subcutaneous injection in mice[10]. 4′-Methyl-1,2-benzanthracene has been shown in one series of tests to be a very weakly active carcinogen towards mouse skin[11].

Several other crystalline substances have been obtained by chromatography of those fractions of the oil which did not react with maleic anhydride. One of these, obtained as colourless needles, m.p. 195–199°, was shown to be triphenylene by mixed melting point with an authentic specimen, m.p. 202–204° [12], by ultra-violet absorption measurements ($\lambda_{max.}$ 249, 257·5, 273, 284, 320, 328, 335 mμ; log ε, 4·93, 5·15, 4·37, 4·32, 2·87, 2·70, 2·72)[13] and by elementary analysis (found: C, 94·6; H, 5·3; calc. for $C_{18}H_{12}$: C, 94·7; H, 5·3 per cent). Another substance, m.p. 252–256°, was identified as chrysene (m.p. 256°)[14] by mixed melting point and ultra-violet absorption ($\lambda_{max.}$ 241, 258, 267; 294, 306·5, 320; 344, 352, 361 mμ; log ε, 4·62, 5·18, 5·36; 4·39, 4·44, 4·43; 3·12, 2·84, 3·10). The infra-red spectrum was identical with that of chrysene, and on vapour-phase chromatography one peak was obtained with retention time similar to that of chrysene. The *s*-trinitrobenzene complex formed yellow needles, m.p. 189°,

not depressed when mixed with an authentic specimen, m.p. 191–193° (lit. m.p. 192–194°)[4].

None of the hydrocarbons reported here has previously been isolated from an uncracked petroleum fraction. Claims have been presented, based on spectroscopic evidence, for the occurrence of chrysene and of 1,2-benzanthracene in the aromatic fraction of a clarified oil[15], and of chrysene, triphenylene and 1,2-benzanthracene in a paraffin wax of unspecified origin[16].

We are indebted to Dr. J. W. Cook for his interest and encouragement, and to Prof. F. Morton, of the Manchester College of Science and Technology, for supplying us with the oil fractions used in the investigation.

W. Carruthers
A. G. Douglas

Medical Research Council
Carcinogenic Substances Research Unit,
Washington Singer Laboratories,
University of Exeter.

[1] Carruthers, W., *J. Chem. Soc.*, 603 (1956).
[2] Jones, R. N., *J. Amer. Chem. Soc.*, **62**, 148 (1940). Badger, G. M., Pearce, R. S., and Pettit, R., *J. Chem. Soc.*, 1112 (1952).
[3] cf. Fuson, N., and Josien, M.-L., *J. Amer. Chem. Soc.*, **78**, 3049 (1956).
[4] Orchin, M., and Woolfolk, E. O., *J. Amer. Chem. Soc.*, **68**, 1727 (1946).
[5] Newman, M. S., and Otsuka, S., *J. Org. Chem.*, **23**, 797 (1958).
[6] Takemura, K. H., Cameron, M. D., and Newman, M. S., *J. Amer. Chem. Soc.*, **75**, 3280 (1953).
[7] Hieger, I., and Woodhouse, D. L., *Brit. J. Cancer*, **6**, 293 (1952).
[8] Hartwell, J. L., *Survey of Compounds which have been Tested for Carcinogenic Activity*, second ed. (United States Government Printing Office, Washington, 1951). Shubik, P., and Hartwell, J. L., Supp. 1 to the *Survey* (1957).
[9] Hadler, H. I., Darchun, V., and Lee, K., *J. Nat. Cancer Inst.*, **23**, 1383 (1959).
[10] Steiner, P. E., and Edgcombe, J. H., *Cancer Res.*, **12**, 657 (1952).
[11] Badger, G. M., *et al.*, *Proc. Roy. Soc.*, **129**, 439 (1940).
[12] Bachman, W. E., and Clarke, H. T., *J. Amer. Chem. Soc.*, **49**, 2089 (1927).
[13] Clar, E., and Lombardi, L., *Ber.*, **65**, 1411 (1932).
[14] Jones, R. C., and Neuworth, M. B., *J. Amer. Chem. Soc.*, **66**, 1497 (1944).
[15] Dietz, W. A., Dudenbostal, B. F., and Priestley, W., Amer. Chem. Soc., Div. Petrol. Chem. Preprints 1, No. 4, *Polycyclic Hydrocarbons*. 117 (1956).
[16] Lijinsky, A., Raha, C. R., and Keeling, J., *Anal. Chem.*, 810 (1961).

Partition Isotherms and Gas-Liquid Chromatography

The various well-known theories of gas-liquid chromatography all assume a linear 'partition' isotherm, that is, the concentration of a solute in the stationary liquid phase is a linear function of the concentration in the gas phase; also the theories are unanimous in predicting symmetrical elution peaks. Asymmetrical peaks, when they occur, are variously ascribed to 'overloading' of the column, lack of attainment of equilibrium, adsorption on the solid support and at the gas-liquid interface, and finally, non-ideal solution behaviour[1]. Non-ideality is taken to mean that the solutions show deviations from Raoult's law. We wish to make two points; first, that partition isotherms are frequently markedly non-linear and, second, that non-ideal solution behaviour is not important in determining elution peak symmetry, but that isotherm shape is. We base our arguments on the results of experiments which were aimed at the determination of partition isotherms by a static method.

Using a modified McBain–Bakr balance we have measured the partition isotherms of a number of vapours in a variety of liquids commonly used as stationary phases in gas-liquid chromatography. The method was as follows: a small sample of 'Celite' coated with the liquid stationary phase was weighed into a small glass bucket and suspended from a silica spring (extension approximately 1 m. per gm.) and the system degassed to a vacuum of 10^{-4}–10^{-5} mm. of mercury. Small charges of the vapour were then admitted to the system and the uptake by the liquid determined by measuring the extension of the spring. In this way we have investigated the solubility of the following vapours at a number of different temperatures: cyclohexane, benzene, dichloromethane, chloroform and carbon tetrachloride in dinonyl phthalate and squalane, and additionally, benzene in benzyl diphenyl, diglycerol and silicone oil. Similar measurements have been made by Everett and Ashworth[2]. Some of the isotherms determined at 30° C. are shown in Fig. 1. With the exception of the system benzene–diglycerol, in which there was negligible uptake of benzene by the diglycerol, even at relatively high pressures, all the isotherms obtained were of the same general shape, that is, all were curved in a direction convex to the concentration axis. This establishes our first point. It is only fair to point out, however, that these isotherms approximate to linearity at low concentrations, hence, provided that only small samples are used in gas-liquid chromatography, symmetrical elution peaks should be obtained. In passing, it is interesting to note that the isotherms curve in the opposite sense to that usually observed in adsorption at a solid surface.

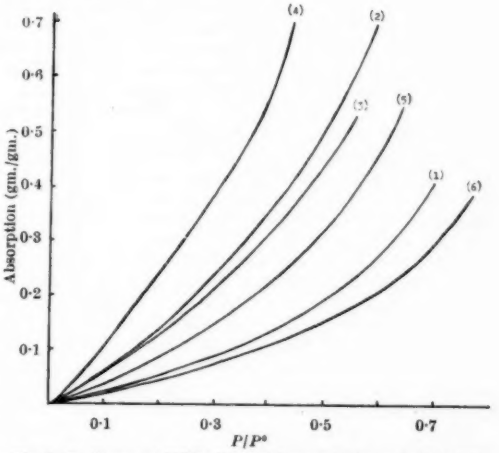

Fig. 1. Isotherms at 30° C. (1) Cyclohexane in dinonyl phthalate; (2) carbon tetrachloride in dinonyl phthalate; (3) dichloromethane in dinonyl phthalate; (4) chloroform in dinonyl phthalate; (5) cyclohexane in squalane; (6) dichloromethane in squalane

It has been shown theoretically by de Vault[3] and experimentally by Gregg and Stock[4] that systems giving rise to isotherms of the shape illustrated in Fig. 1 will, if used in chromatography, tend to give elution peaks with diffuse front boundaries ('fronting') and sharp tails, and there is no doubt that such peaks are frequently observed in gas-liquid chromatography when rather large samples are used (as may be necessary when a comparatively insensitive detector is employed). Now Raoult's law defines an ideal solution as one which gives a straight line when the partial vapour pressure of a component is plotted against its mole fraction, x. (In the systems under consideration only one of the components exerts an appreciable vapour pressure; that of the stationary

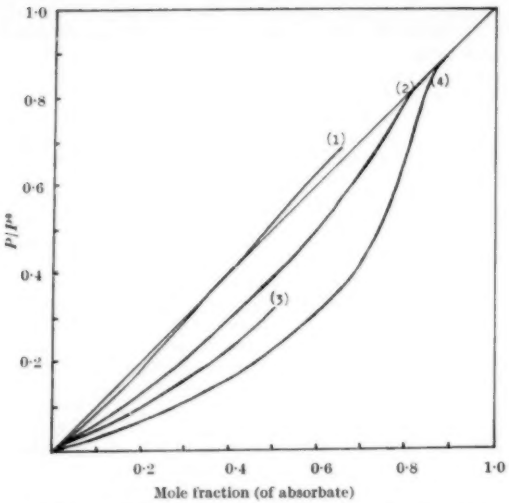

Fig. 2. Deviation from Raoult's law at 30° C., all in dinonyl phthalate. (1) Cyclohexane; (2) carbon tetrachloride; (3) dichloromethane; (4) chloroform

liquid can be neglected.) If we now replot the isotherms involving dinonyl phthalate in Fig. 2 using mole fractions instead of concentrations, we observe that three of the systems show negative deviations from Raoult's law and one shows both a positive and a negative deviation, in spite of the fact that the corresponding isotherms are all the same general shape. It can easily be ascertained that systems which obey Raoult's law or give positive deviations will still give curved isotherms of the type illustrated. To obtain isotherms which are curved in the opposite sense, that is, are concave to the concentration axis, and which would therefore give rise to tailing, the most pronounced negative deviations from Raoult's law are necessary. Such a system has never been observed by us. We are therefore of the opinion that 'fronting' is an inherent tendency in gas-liquid chromatography, but will not be marked, or even noticeable, when low concentrations of the solute are used. Tailing is seldom, if ever, caused by deviations from ideality of the gas-liquid system, and is most probably due to the adsorption on the solid support, or perhaps at the gas-liquid interface.

Finally, we would like to record that our results for the system showing both positive and negative deviations from Raoult's law, namely, cyclohexane–dinonyl phthalate, confirm the measurements and predictions of Everett and Ashworth[2]. Very careful measurements were made to establish the deviation at low concentrations of cyclohexane, and the relevant data are given in Table 1. Further investigations have shown that this type of system is by no means uncommon in gas-liquid chromatography.

Table 1. Results for the System: Cyclohexane and Dinonyl Phthalate at 30° C.

P/P^0	X	$\log_{10} P/P^0X$	$\frac{B(P^0-P)}{2{\cdot}303\,R.T}$	$\log_{10}\gamma^0/f$
0·02628	0·02841	$\bar{1}$·9661	0·0041	$\bar{1}$·9701
0·06483	0·06904	$\bar{1}$·9726	0·0039	$\bar{1}$·9765
0·11903	0·12504	$\bar{1}$·9786	0·0037	$\bar{1}$·9823
0·20486	0·21123	$\bar{1}$·9867	0·0033	$\bar{1}$·9900
0·25321	0·25772	$\bar{1}$·9923	0·0031	$\bar{1}$·9954
0·30065	0·30318	$\bar{1}$·9964	0·0029	$\bar{1}$·9993
0·35461	0·35424	0·0004	0·0027	0·0031
0·41186	0·40826	0·0033	0·0025	0·0057
0·48327	0·47396	0·0086	0·0021	0·0107
0·56548	0·54729	0·0140	0·0018	0·0158
0·66598	0·63829	0·0182	0·0014	0·0196

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G. F. Freeguard
R. Stock

Chemistry Department,
College of Technology,
Byrom Street,
Liverpool, 3.

[1] Porter, P. E., Deal, C. H., and Stross, F. H., *J. Amer. Chem. Soc.*, **78**, 2999 (1956).
[2] Everett, D. H., and Ashworth, A. J., *Trans. Farad. Soc.*, **56**, 1609 (1960).
[3] de Vault, D., *J. Amer. Chem. Soc.*, **65**, 532 (1943).
[4] Gregg, S. J., and Stock, R., *Gas Chromatography*, edit. by Desty D. H., 90 (Butterworths, London, 1958).

A Cyclic Tetramer of Chloral

Treatment of chloral or its hydrate with concentrated sulphuric acid yields a number of polymeric materials which were identified by Chattaway and Kellett[1] and studied by Novak and Whalley[2]. From the mixture obtained in this Laboratory by treating some 300–400 gm. of chloral hydrate, about 2 gm. (0·5 per cent) of a new polymeric compound was isolated as a white crystalline solid (m.p. 219–220° corr.). It crystallized from absolute ethanol as short needles slightly less soluble in this solvent than chloralide or α-parachloral. The compound is very soluble in acetone, pyridine and ether; moderately soluble in boiling ethanol; slightly soluble in carbon disulphide, carbon tetrachloride and cold ethanol and insoluble in water. Boiling with an aqueous ethanol-20 per cent sodium hydroxide solution liberates chloroform and formic acid. Further work gave the following results: Analysis, calculated for $(CCl_3–CHO)_n$: C, 16·28; H, 0·68; Cl, 72·17. Found: C, 16·43; H, 0·73; Cl, 71·78. Molecular weight (Rast), calculated for $(CCl_3–CHO)_4$, 589·5. Found: 614, 616, 571 (average 600·3). Infra-red spectrum: the compound was examined in 'Nujol' mull (0·025 mm.) and in saturated (18° C.) carbon tetrachloride and carbon disulphide solutions (1 mm.), on a model 137 'Infracord' spectrophotometer (with sodium chloride prism). The bands observed are recorded in Table 1.

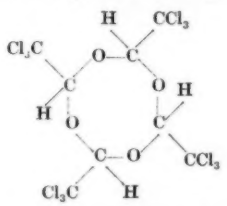
Table 1
Wave-length (μ)

Solid	Solution
3·49, weak	3·49, weak
6·60, medium	6·60, medium
7·23, medium	7·23, medium
7·50, medium	
7·50, medium	7·52, strong
7·57, strong	
	8·55, sharp
8·60, very strong	
	8·75, very strong
8·78, very strong	
9·22, strong	
9·37 strong	
	9·40, medium
	9·50, medium
9·80, very strong	9·80, strong
10·30, medium	
11·78, very strong	11·78, very strong
12·30, very strong	12·30, very strong
12·55, sharp	
13·90, sharp	13·90, sharp
14·10, strong	14·10, strong
	14·50, strong
15·00, strong	

The results in Table 1 indicate: (*a*) the absence of hydroxyl and carbonyl groups; (*b*) the presence of a cyclic ether linkage due to the series of bands in

the 950–1,200 cm.$^{-1}$ (8·3–10·5μ) and 800–860 cm.$^{-1}$ (11·6–12·5μ) regions; and (*c*) a shift towards lower frequencies as compared with bands of other known cyclic ethers[3]. It is a well-established fact that there is a shift to lower frequencies whenever there is an increase in ring-size among cyclic ethers.

These results are consistent with the existence of a cyclic tetramer having the following formula:



A report on the structure of this compound, 2,4,6,8-tetrakis-(trichloromethyl)-1,3,5,7-tetroxocane, will be published elsewhere.

I am indebted to Dr. Oscar H. Saggese of this Department for his interpretation of the infra-red spectra.

MÁXIMO BARÓN

Laboratorio de Investigaciones,
Departamento Científico,
Atanor S.A.M.,
Munro, Pcia Buenos Aires.

[1] Chattaway, F. D., and Kellett, E. G., *J. Chem. Soc.*, 2709 (1928).
[2] Novak, A., and Whalley, E., *Canad. J. Chem.*, **36**, 1166 (1958).
[3] Barrow, M. G., and Scott, S., *J. Amer. Chem. Soc.*, **75**, 1175 (1953).

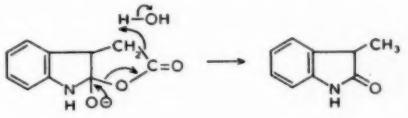
BIOCHEMISTRY

Oxidative Decarboxylation of Indole-3-acetic Acid by Mangani-Versene and by Wheat Leaf Enzyme

THERE is little agreement concerning either the pathway or the final products of the enzymic oxidation of heteroauxin[1]. The stoichiometry of the oxidative decarboxylation seems to be reasonably well established[1], one mole of oxygen being taken up and one of carbon dioxide evolved for every mole of indole-3-acetic acid (IAA) reacting. Galston[2] reported that no indole-3-aldehyde was formed when IAA was destroyed by a pea brei (see, however, Racusen[3]); two principal products were formed with R_F very close to that of *o*-aminoacetophenone (I) and *o*-formamidoacetophenone (II) and giving similar colour reactions. On the basis of a ferric chloride test, Galston concluded that these compounds were hydroxylated derivatives of (I) and (II). Indole-3-aldehyde has an R_F intermediate between those of (I) and (II) and does give a brownish-pink colour with ferric chloride; the colour reported by Galston is undoubtedly due to the spot for the indolealdehyde overlapping on to the other two spots. It also seems most unlikely that the R_F of phenolic derivatives of both (I) and (II) should be the same as those of (I) and (II). Finally, should such phenols be formed, the uptake of oxygen should be at least 1·5 times that actually reported. Using an oxidizing enzyme from *Omphalia flavida*, Stowe *et al.*[4] rejected the possibility that (I), (II) or indolealdehyde was formed in any amount and suggested that the product (not isolated) might be 3-methyldioxindole. From their chemical evidence it seems rather unlikely that their product was 3-methyldioxindole. Most of their observations can be explained on the basis of our present results. With *Lupinus* seedlings Stutz[5] obtained a number of products (one of them being indolealdehyde) chromatographically similar to those obtained by Galston[2]. Using substituted 3-indoleacetic acids Stutz concluded that the ring system (probably C_2) and not the side chain was the primary site of dehydrogenation.

MacLachlan and Waygood[6] reported that the oxidative decarboxylation of IAA by mangani-versene in a phosphate buffer at *p*H 6 gave a product which appeared to be chromatographically identical with that obtained by the oxidation of IAA in the presence of wheat leaf enzyme, or by using a peroxidase in the presence of manganese ions and 2,4-dichlorophenol. Waygood *et al.*[7] also suggested a radical chain mechanism for this reaction involving the formation of a skatyl radical leading to a product $C_9H_9O_2N$ (not established) (cf. Hewitt[8]). No pure compound was actually isolated, all the conclusions being based on the rates of absorption of oxygen and evolution of carbon dioxide.

We have now oxidized IAA with oxygen in the presence of mangani-versene at *p*H 6 and isolated the basic, neutral and acidic fractions. The neutral fraction (57·5 per cent based on IAA) was analysed using paper, column, and vapour phase chromatography. By preparative vapour phase chromatography two compounds could be identified (in all cases here by analysis, melting points, mixed melting points with authentic samples, R_F values, and infra-red spectra) as *o*-aminoacetophenone (I) (very small amounts) and *o*-formamidoacetophenone (II) (9–13 per cent). Column chromatography of the neutral fraction gave *o*-aminoacetophenone (10–13 per cent) (*o*-formamidoacetophenone is hydrolysed to (I) under these conditions), followed by a small amount of unidentified crystals, melting point 164–165° (no C = O bands in the infra-red). Indole-3-aldehyde was eluted next (12·3 per cent crude yield), and, finally, a low-melting solid (25 per cent) which was chiefly 3-methyloxindole (III). It would thus seem as though all the postulated modes of primary attack are occurring in this system. Phenolic products are probably also formed (cf. ref. 9) and are present in the acidic fraction together with some unreacted IAA, but this was not investigated. Formation of 3-methyloxindole probably proceeds by primary attack at C_2 of the indole ring followed by the assisted decarboxylation of a cyclic intermediate such as (IV):



(IV) (III)

The formation of (I) and (II) is analogous to the conversion of tryptophan to kynurenine and probably involves attack at C_3 (ref. 10). The formation of indole-3-aldehyde necessitates primary attack at C_α of the side-chain.

In the case of the enzymic oxidation products[6,7] (reaction mixtures kindly supplied by Dr. E. R. Waygood) neither (I) nor (II) could be detected. Column chromatography on alumina led to the isolation in low yield of 3-methyloxindole mainly together with indole-3-aldehyde.

A preliminary investigation (with Mr. K. A. H. Adams) of the mangani-versene catalysed oxidation of

IAA at *p*H 4 gave a coloured product (melting point 160° with decomposition) which was very difficult to purify and the molecular weight of which was approximately 576. This compound gave only one spot on paper chromatography, but decomposed on alumina into three fractions. A small amount of the same product also precipitates out in the *p*H 6 reaction.

We wish to thank Prof. Waygood for generous supplies of enzymic oxidation mixtures and the National Research Council of Canada (Prairie Regional Committee) for financial support.

R. A. Abramovitch
K. S. Ahmed

Department of Chemistry,
University of Saskatchewan,
Saskatoon.

[1] Ray, P. M., *Ann. Rev. Plant Physiol.*, **9**, 81 (1958).
[2] Galston, A. W., in *The Chemistry and Mode of Action of Plant Growth Substances*, edit. by Wain, R. L., and Wightman, F., 219 (Butterworths, 1956).
[3] Racusen, D., *Arch. Biochem. Biophys.*, **58**, 508 (1955).
[4] Stowe, B. B., Ray, P. M., and Thiman, K. V., *Eighth Cong. Intern. Botan., Paris, Rappa. et communs.*, Suppl. Vol., 135 (1954). Ray, P. M., and Thimann, K. V., *Arch. Biochem. Biophys.*, **64**, 175 (1956).
[5] Stutz, R. E., *Plant Physiol.*, **33**, 207 (1958).
[6] MacLachlan, G. A., and Waygood, E. R., *Physiol. Plantarum*, **9**, 321 (1956); *Canad. J. Biochem. Physiol.*, **34**, 1233 (1956).
[7] Waygood, E. R., Oaks, A., and MacLachlan, G. A., *Canad. J. Bot.*, **34**, 54, 905 (1956).
[8] Hewitt, E. J., *Nature*, **180**, 1020 (1957).
[9] Horning, E. C., Sweeley, C. C., Dalgliesh, E. C., and Kelly, W., *Biochim. Biophys. Acta*, **32**, 566 (1959).
[10] Elderfield, R. C., *Heterocyclic Compounds*, **3**, 69 (John Wiley and Sons, New York, 1952).

Complement-fixing Antibodies to Human Growth Hormone and Sheep Interstitial Cell Stimulating Hormone

Previous reports from this Laboratory[1-3] have shown that human growth hormone (HGH) and sheep interstitial cell stimulating hormone (ICSH) produce, in rabbits, precipitin antibodies that are specific to the respective hormones. Immunochemical techniques have been used to characterize these hormones as well as to perform quantitative determinations of small quantities of the hormone. Hæmagglutination inhibition has also been used to study and detect HGH in human serum[4]. The purpose of the present investigation was to study HGH and sheep ICSH by another immunochemical reaction, namely, complement fixation. It may be noted that the complement fixation method is capable of determining quantitatively as little as 0·01 μgm. of the hormone and is an objective procedure which is more reproducible than the hæmagglutination technique.

Rabbit antisera to HGH and sheep ICSH were produced and the γ-globulin was obtained as previously described[2,3]. The procedure for the complement fixation tests was modified from that described by Kabat and Mayer[5]. For a typical experiment, 0·02 μgm. HGH was dissolved in 0·5 ml. saline solution and 20 μgm. anti-HGH γ-globulin plus 500 μgm. lyophilized guinea pig serum in 0·5 ml. veronal buffer of *p*H 7·3 were added. With HGH, complement fixation was carried out by incubation for an initial interval of 20 min. at 37° C., followed by further incubation overnight in the refrigerator at 4° C. In the case of ICSH, incubation for 1 hr. at 37° C. was sufficient.

For the determination of uncombined complement, a suspension of hæmolysin-sensitized sheep red blood cells was added and the contents of the tubes were allowed to incubate for 1 hr. at 37° C. The non-hæmolysed intact red blood cells were removed by centrifugation; the clear supernatant fluid was diluted to the proper concentration for estimation of hæmoglobin concentration at 540 mμ in a Beckman spectrophotometer, model DU. Increased complement fixation resulted in less hæmolysis and, consequently, a lower optical density. Since the quantity of complement added is a critical step for reproducible results, a single lot of complement (Difco) with periodic titration was used in this work. The amount of complement used was sufficient to lyse about 60 per cent of the sheep red blood cells in the tubes containing the lowest concentration of antigen.

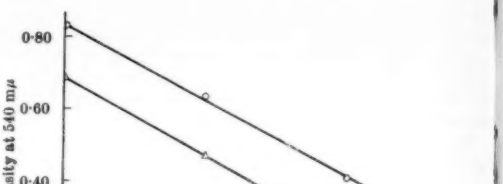


Fig. 1. Amount of complement fixed as a function of human growth hormone (HGH) and sheep interstitial cell stimulating hormone (ICSH) concentrations. Δ, HGH; ○, sheep ICSH

The standard curves, which were obtained by varying the concentration of the hormone while keeping constant the amounts of all other components of the system, are shown in Fig. 1. A linear response was observed up to 0·06 μgm. of ovine ICSH and 0·04 μgm. of HGH. The complement fixation tests were found to be reproducible if care was used in all phases of the test. As a routine practice, it is always necessary to use a sufficient number of controls to detect anti-complement or synergistic activity and non-specific hæmolysis.

The amount of complement fixed as a linear function of the hormone concentration suggested that the complement fixation reaction could be used as a quantitative method for determining HGH and ovine ICSH. This technique has thus been employed for estimation of hormonal content in the pituitary gland. Fresh pituitaries were homogenized in saline solution and the clear supernatant fluid was used. The HGH content of two glands was found to be 21·3 and 31·8 μgm. per mgm. of fresh tissue by complement fixation and 19·3 and 36·1 μgm. per mgm. by quantitative precipitin tests[2]. By complement fixation the HGH in three acetone-dried glands was estimated as 4·4 μgm. per mgm. of dry tissue and 4·1 μgm. per mgm. by the precipitin test[2]. These results were in good agreement with values estimated on the basis of the results of the tibia test in hypophysectomized rats[6].

Similar studies were carried out for the estimation of the ICSH content in sheep pituitaries. It was found that two sheep pituitaries contained 0·9 μgm. and 0·8 μgm., respectively, of ICSH per mgm. of fresh tissue when the content was determined by complement fixation and 1·0 μgm. and 0·9 μgm., respectively, of ICSH per mgm. by the precipitin test[3].

The results of this investigation demonstrate that antibodies, prepared from rabbit antisera, to highly purified preparations of human growth hormone and sheep ICSH fix complement when allowed to react with the respective antigens. On a quantitative basis, the complement fixation reaction will detect about one-hundredth of the quantity of hormone measured by precipitin reactions. The close agreement of the values obtained by the technique of complement fixation with those obtained by quantitative precipitin tests indicates that complement fixation can be used as a quantitative immunochemical technique for measuring minute quantities of HGH and sheep ICSH.

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ALLEN TRENKLE
N. R. MOUDGAL
KATY K. SADRI
CHOH HAO LI

Hormone Research Laboratory,
University of California,
Berkeley, California.

[1] Hayashida, T., and Li, C. H., *Science*, **128**, 1276 (1958).
[2] Li, C. H., Moudgal, N. R., and Papkoff, H., *J. Biol. Chem.*, **235**, 1038 (1960).
[3] Moudgal, N. R., and Li, C. H., *Arch. Biochem. and Biophys.* (In the press).
[4] Read, C. H., and Bryan, G. T., in *Ciba Foundation Colloquia on Endocrinology*, **13**, 68 (1960).
[5] Kabat, E. A., and Mayer, M. M., *Experimental Immunochemistry*, 97 (Charles Thomas, Springfield, Illinois, 1948).
[6] Greenspan, F. S., Li, C. H., Simpson, M. E., and Evans, H. M., *Endocrinol.*, **45**, 455 (1949).

Effect of Riboflavin on Liver Histomucoid

Isenberg and Szent-Györgyi[1] studied the reaction between tryptophan and tryptophan metabolites and riboflavin. It was observed that free tryptophan or this amino-acid in proteins formed a complex with riboflavin. The vitamin molecule takes up one electron from tryptophan and a semiquinoid riboflavin free radical is formed. Those authors speculated that this complex occurs *in vivo*. Since it was established by Winzler *et al.*[2] that seromucoid contains tryptophan, and in view of the hepatic origin of that fraction[3], experiments were conducted by us to investigate a possible effect of riboflavin on liver histomucoid *in vivo*.

Male Wistar rats with mean body weight of 135 gm. were injected intraperitoneally with a freshly prepared riboflavin solution (20 mgm./100 ml. of physiological saline). The dose used was 0·2 mgm. of riboflavin per 100 gm. of body weight. One group of animals received only one injection and the other was treated with a daily dose for 7 days. Control rats were injected with the same volume of the saline solution. All the animals were fed *ad libitum* a standard balanced diet, and liver histomucoid determinations were carried out, by the method previously reported[4], 24 hr. after the last injection. A summary of the results is shown in Table 1.

Table 1 clearly shows that in both riboflavin groups lowered levels of liver histomucoids were observed. The differences between riboflavin-injected rats and the controls were statistically significant (1 treatment: $t = 3{\cdot}733$, $P < 0{\cdot}01$; 7 treatments: $t = 4{\cdot}711$, $P < 0{\cdot}001$). From the results presented it is obvious that one dose of riboflavin is sufficient for the production of the full effect.

Table 1. LIVER HISTOMUCOID IN RATS TREATED WITH RIBOFLAVIN

Treatment	No. of injections	No. of rats	Histomucoid (mgm. hexose/100 gm. acetone powder) Range	Mean ± S.D.
Saline	1	6	105–191	152 ± 36
Riboflavin	1	6	89–108	96 ± 7
Saline	7	8	110–187	146 ± 24
Riboflavin	7	11	67–127	97 ± 20

Seromucoid determinations[3] in the same animals were carried out and no differences were disclosed between control and riboflavin-treated rats. This result warrants the conclusion that the lowered liver histomucoid observed was not produced by a mobilization of mucoproteins from that organ to the plasma.

On the other hand, incubation of extracts from liver acetone powders[4] or liver homogenates in physiological saline with riboflavin at 37° C. up to 3 hr. gave no change in the histomucoid-level.

Further investigations are being undertaken to elucidate the mode of the action of riboflavin on liver histomucoids.

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LUIZ A. ABREU
R. RAPOSO ABREU

Biochemical Laboratory,
Instituto Oswaldo Cruz,
Rio de Janeiro.

[1] Isenberg, I., and Szent-Györgyi, A., *Proc. U.S. Nat. Acad. Sci.*, **44**, 857 (1958).
[2] Winzler, R. J., Devor, A. W., Mehl, J. W., and Smyth, I. M., *J. Clin. Invest.*, **27**, 609 (1948).
[3] Winzler, R. J., *Methods Biochem. Anal.*, **2**, 279, edit. by Glick, D. (Interscience, 1955).
[4] Abreu, L. A., and Abreu, R. R., *Nature*, **184**, 2016 (1959).

D-Penicillamine as an Antidote to 8-Hydroxyquinoline and Alloxan

OWING to the chelate-forming effect of its —SH and NH_2 radicals, DL-penicillamine has hitherto been used for removing copper deposited in brain tissues in cases of Wilson's disease and for preventing intoxication caused by heavy metals[1]. It is, however, undesirable to use L-penicillamine as a remedy or a preventive, because of its strong toxicity which causes acute and chronic deficiency of vitamin B_6 (refs. 2–4).

D-penicillamine, on the other hand, has no toxicity. We have examined its preventive and remedial effects on intoxication by 8-hydroxyquinoline and alloxane, which are diabetogenic agents.

0·03 mgm./gm. of body-weight was determined as the lethal dose of 8-hydroxyquinoline for mice 10 hr. after intramuscular injection. 0·2 mgm./gm. of D-penicillamine, injected 5 min. before the injection of lethal doses of 8-hydroxyquinoline, prevented death of 60 per cent of the experimental animals or showed effects in retarding death. Injection of 0·5 mgm./gm. of D-penicillamine prevented death completely but could not prevent toxic symptoms

Table 1

No. of Mice	10	10	10	10	10
D-Penicillamine mgm./body-weight (gm.)	1·0	0	0·2	0·5	1·0
8-Hydroxyquinoline mgm./body-weight (gm.)	0	0·03	0·03	0·03	0·03
Toxic symptoms	—	+ + +	+ +	+	—
No. of mice dead	0	10	4	0	0

Table 2

No. of mice	10	10	10	10	10	3
Tested compounds mgm./body-weight (gm.)	—	D-penicillamine 1·0	DL-cystine 1·0	Vitamin B_6 1·0	Glucose 1·0	Vitamin B_{12} 0·15
Alloxan mgm./body-weight (gm.)	0·45	0·45	0·45	0·45	0·45	0·45
Toxic symptoms	+ + +	+	+ + +	+ + +	+ + +	+ +
No. of mice dead within 15 hr.	10	3	10	10	10	2

such as dyspnœa, tremor and paralysis. Injection of D-penicillamine (1·0 mgm./gm.), however, was successful in preventing death as well as all toxic symptoms (Table 1). Further, D-penicillamine injection in the same amount, 1 hr. before the lethal dose of 8-hydroxyquinoline, could also save the animals from death.

On the other hand, injection of 1 mgm./gm. of vitamin B_6, DL-cystein (hydrochloride) or glucose or of 0·15 mgm./gm. of vitamin B_{12} 5 min. before the injection of lethal doses of 8-hydroxyquinoline could not prevent death in these animals.

1·0 mgm./gm. D-penicillamine injection 5 min. before the injection of alloxane in the lethal doses (0·45 mgm./gm.) retarded death, but DL-cystein, vitamin B_6, glucose or vitamin B_{12}, injected in the amount mentioned above, could not prevent or retard death (Table 2).

8-Hydroxyquinoline injection in an amount less than the lethal dose caused the blood sugar in mice to increase by approximately 100 per cent in 1–3 hr. and, thereafter, in 6–16 hr., to decrease by approximately 40 per cent, during which time some of the mice died of intoxication. The blood sugar returned to the normal level in 24 hr. and, thereafter, in most cases showed slightly higher values than the normal. In order to test the influence of D-penicillamine upon the changes of blood sugar values brought about by 8-hydroxyquinoline, 1·0 mgm./gm. of D-penicillamine was injected 5 min. before 8-hydroxyquinoline injection, but no influence whatever was observed. Accordingly, it is considered that death caused by 8-hydroxyquinoline and the antidotal effect of D-penicillamine against 8-hydroxyquinoline has nothing to do with the changes of blood sugar values.

With regard to the mechanism of the preventive action of D-penicillamine, we are still conducting tests.

Makoto Matsuda
Katashi Makino

Department of Biochemistry,
The Tokyo Jikei-kai School of Medicine,
Minato-ku, Tokyo.

[1] Walshe, J. M., *Amer. J. Med.*, **21**, 487 (1956).
Wilson, J. E., and du Vigneaud, V., *J. Biol. Chem.*, **184**, 63 (1950).
[2] Kuchinskas, E. J., and du Vigneaud, V., *Arch. Biochem. Biophys.*, **66**, 1 (1957).
[4] Kuchinskas, E. J., Horvath, A., and du Vigneaud, V., *Arch. Biochem. Biophys.*, **68**, 69 (1957).

Preparation and Determination of Sulphosalicylo-soluble Proteins using Tannin

In previous papers[1–3], regeneration of proteins from insoluble protein-tannin compounds (PT) after treatment with caffeine, without loss of physicochemical and biological properties, was reported. The present communication is concerned with determination and preparation of sulphosalicylo-soluble proteins (SSP). The proteins belonging to that group are, for example, gonadotropins, thyreotropin, orosomucoid, haptoglobin, submaxillary mucoprotein, ovomucin, lysozyme, uromucoid and acid mucopolysaccharide-containing proteins.

In this communication the turbidimetric micro-method[4] is applied to estimation of both groups of proteins, soluble and insoluble ones, in sulphosalicylic acid (SSA). It has been shown that 10–100 μgm. of dried gelatin, lysozyme, orosomucoid or mucin, dissolved in 1 ml. of 0·9 per cent sodium chloride or 0·15 *M* SSA, produce with tannin reagent similar turbidity as hæmoglobin, fibrinogen or egg albumin dissolved in 1 ml. of 0·9 per cent sodium chloride. Under conditions of the method (*p*H about 0, excess of tannin with reference to weight of protein), irreversible PT compounds are formed. Turbidities, measured as optical density, are directly proportional to the weight of proteins and do not depend on molecular weight, nitrogen content or on presence of a non-protein moiety. In this way it is possible to apply the same criterion for the determination of proteins which are soluble or insoluble in SSA, and an appropriate conversion factor should be used.

It has been shown that as well as previously investigated proteins, orosomucoid and lysozyme form reversible PT compounds at their isoelectric points and below, at a definite ratio of weight of the reactants[5]. For the precipitation of proteins at *p*H about 1(0·15 *M* SSA), 400 mgm. of tannin should be added to each 100 ml. of the solution and then an amount of tannin equal to protein content. The precipitate is centrifuged and washed with water until it is free from SSA. Lysozyme and orosomucoid, after regeneration with caffeine, retain their electrophoretic mobility and solubility in SSA unchanged. SSP can be recovered with 90 per cent yield.

Concentrations of total protein as well as that remaining in SSA filtrate have been determined in different biological materials. For example, in 100 ml. of gastric juice, normal serum or urine (without uromucoid), 0·2, 6·0 and 0·005 gm. of protein and 0·2, 0·07 and 0·002 gm. of SSP, respectively, were found[1]. Very small amounts of SSP in normal serum and urine require to be concentrated before paper electrophoresis. For that purpose, the tannin and caffeine procedure was applied[1]. SSP of serum had been concentrated 50-fold and those of urine 1,000-fold, and they were separated afterwards by paper electrophoresis. The results of one experiment in which both

Table 1. Content of the Sulphosalicylo-soluble Proteins (SSP) in the Electrophoretic Fractions of Normal Human Serum and Urine

Fraction	Serum Protein gm./100 ml.	Serum SSP gm./100 ml.	Serum SSP Per cent of fraction	Urine* Protein gm./100 ml.	Urine* SSP gm./100 ml.	Urine* SSP Per cent of fraction
Albumin	3·40	0·0045	0·13	0·00073	0·00013	18·0
α_1-Globulin	0·34	0·0470	13·90	0·00048	0·00034	72·6
α_2-Globulin	0·72	0·0097	1·34	0·00054	0·00016	28·8
β-Globulin	0·92	0·0066	0·72	0·00158	0·00043	26·0
γ-Globulin	1·14	0·0142	1·25	0·00048	0·00029	60·5
Protein without electrophoresis	6·6	0·0820	1·24	0·00381	0·00135	35·5

* Urine without uromucoid of Tamm and Horsfall.

Paper electrophoresis in veronal buffer of *p*H 8·6 and ionic strength 0·09 (ref. 8). Protein was concentrated by the tannin–caffeine procedure (ref. 2). Amount of SSP in 100 ml. of serum, amount of protein in 100 ml. of urine and SSP in 100 ml. of urine were, after the concentration procedure, 3·1, 1·8 and 2 gm., respectively. The results are obtained by dividing the obtained values by degree of concentration.

the protein and regenerated SSP of normal human serum and regenerated protein and SSP of normal urine were determined after paper electrophoresis are shown in Table 1. The values obtained for orosomucoid of normal human serum were strikingly similar to those of Winzler[6].

Electrophoretic paper strips of regenerated proteins and SSP of normal urine were stained for acid mucopolysaccharides with alcian blue according to Heremans and Vaerman[7]. Three fractions were found with anodic mobility greater than albumin even though chondroitic acid and heparin are not precipitable with tannin. The presence of acid mucopolysaccharides proves that they are precipitated together with the protein component and are then dissociating during electrophoresis.

W. Mejbaum-Katzenellenbogen
W. Dobryszycka
B. Morawiecka

Department of Biochemistry,
Medical School in Wrocław,
University of Wrocław,
Poland.

[1] Mejbaum-Katzenellenbogen, W., *Acta Biochem. Polon.*, **6**, 375, 385 (1959).
[2] Mejbaum-Katzenellenbogen, W., Dobryszycka, W., Bogusławska-Jaworska, J., and Morawiecka, B., *Nature*, **184**, 1799 (1959).
[3] Mejbaum-Katzenellenbogen, W., and Morawiecka, B., *Acta Biochem. Polon.*, **6**, 453 (1959).
[4] Mejbaum-Katzenellenbogen, W., *Acta Biochem. Polon.*, **2**, 279 (1955).
[5] Mejbaum-Katzenellenbogen, W., Morawiecka, B., and Dobryszycka, W., *Acta Biochem. Polon.*, **7**, 401 (1960).
[6] Winzler, R. J., *Methods of Biochemical Analysis*, edit. by Glick, D., **2**, 279 (Interscience Pub., Inc., New York, 1955).
[7] Heremans, J. F., and Vaerman, J. P., *Clin. Chim. Acta*, **3**, 430 (1958).
[8] Mejbaum-Katzenellenbogen, W., and Dobryszycka, W., *Clin. Chim. Acta*, **4**, 515 (1959).

Comparative *in vitro* Activities of Phosphorylase *b* and Cytochrome Oxidase in Preparations from Two Ox Muscles

It is generally observed that glycolysis in mammalian muscles ceases when the *p*H falls to 5·4–5·5 and that this *p*H is usually reached unless there is insufficient glycogen present in the muscle at the moment of death. In some muscles, however, as much as 1 per cent of glycogen may remain unconverted to lactic acid at an ultimate *p*H of 5·9–6·0 [1,2]. One such muscle is ox *sternocephalicus*. It has been shown that during post-mortem glycolysis in this muscle the length of the external glycogen chains diminishes significantly. Thus, while the mean number of glucose residues in external chains of glycogen molecules for ox *sternocephalicus* glycogen is 12 before the onset of *rigor mortis* (pre-rigor), the mean number of glucose residues for the residual glycogen[3] (post-rigor) is 9: the latter is apparently insusceptible to further glycolysis *in situ*. In these circumstances, it was of interest to compare the behaviour of the glycogen isolated *post rigor* from ox *sternocephalicus* when exposed *in vitro* to the action of phosphorylase *b* preparations from ox *sternocephalicus* and *psoas major* muscles respectively. The rate of *in vitro* breakdown of some other muscle glycogens by these phosphorylases has also been considered.

The methods for preparing pre- and post-rigor samples of muscle glycogen have been described earlier[3]. Phosphorylase *b* was prepared by the method of Fischer and Krebs[4], and its enzymic activity in forming glucose-1-phosphate from glycogen was measured as described by Illingworth and Cori[5]. The activity of phosphorylase *b* prepared from ox *psoas major* muscles against pre-rigor glycogen from this same source has been given an arbitrary value of 100. Findings are summarized in Table 1.

Table 1. Relative *in vitro* Activity* of Ox Phosphorylase *b* from Two Ox Muscles with Various Samples of Muscle Glycogen †

Source of phosphorylase *b*‡ preparation	Source of glycogen: *Psoas major* (ox) pre-rigor	*Psoas major* (ox) post-rigor	*Sterno-cephalicus* (ox) post-rigor	Heart (horse) pre-rigor	Heart (horse) post-rigor
Psoas major§	100	100	80	130	95
Sterno-cephalicus§	100	110	75	145	115

* Rate of glucose-1-phosphate formation in a reaction mixture of 0·2 m*M* adenylic acid, 1,000 mgm. per cent glycogen, 25 mgm. per cent inorganic phosphate and 5 mgm. per cent phosphorylase *b* protein in 0·07 *M* glycerophosphate and 0·06 *M* cysteine buffer, *p*H 6·8. Activity of *psoas major* phosphorylase *b* with *psoas major* (pre-rigor) glycogen taken as 100 = 850 units/mgm. protein.
† As prepared by Lawrie *et al.* (ref. 3).
‡ As prepared by Fischer and Krebs (ref. 4).
§ Mean values of three determinations except for *psoas major* (post-rigor).

It is clear that phosphorylase *b* preparations from *psoas major* and *sternocephalicus* muscles of the ox break down a given glycogen at comparable rates. Different glycogens, however, are attacked at markedly different rates by both phosphorylase *b* preparations. Thus pre-rigor glycogen from horse heart is broken down at about twice the rate of post-rigor glycogen from ox *sternocephalicus* (Table 1).

The results indicate that post-rigor glycogen from ox *sternocephalicus* is broken down *in vitro* by phosphorylase *b* prepared from this same muscle. This suggests that its apparent insusceptibility to attack during post-mortem glycolysis cannot be entirely explained by those structural changes in the molecule represented by the shortening of the mean number of glucose residues in external chains of glycogen molecules. In this context it may be mentioned that pre- and post-rigor glycogens from horse diaphragm and *psoas* muscles have similar mean number of glucose residues, yet in both muscles there is 0·6–1 per cent of residual glycogen at ultimate *p*H values above 5·85 [3]. On one hand it could mean that phosphorylase is more readily inactivated during post-mortem glycolysis in ox *sternocephalicus* than in ox *psoas*. On the other hand, it could signify that, apart from the magnitude of the store of muscle glycogen, intracellular organization is a factor in determining the extent of post-mortem glycolysis. It is not inconceivable that glycogen is functionally differentiated *in vivo* and that there may be concomitant specificity of localization. Thus, in muscles such as ox *sternocephalicus*, some of the glycogen present *in vivo* may be inaccessible to attack under the conditions prevailing in the immediate post-mortem period.

The relatively high activity of phosphorylase *b* with pre-rigor glycogen from horse heart is compatible with the rapidity of glycolysis in the latter post mortem[6] and under anaerobic conditions *in vivo*[7]. Pre- and post-rigor glycogens from ox *psoas major* are apparently converted into glucose-1-phosphate at much the same rate by phosphorylase *b* preparations *in vitro*. This accords with their similar branching structure[3] and with the fact that no residual glycogen is found in this muscle above an ultimate *p*H of 5·4–5·5 (ref. 8).

The potential respiratory capacity of the ox *sternocephalicus* was compared with that of the *psoas major* by determining cytochrome oxidase in enzyme preparations[10] made from each (Table 2). Both the concentration of enzyme solids, and their

Table 2. Comparative Data on Cytochrome Oxidase Activity in Enzyme Preparations from Ox *psoas major* and Ox *sternocephalicus* Muscles

Source of enzyme preparation	Cytochrome oxidase* Qo_2 mean and *S.E.*	Mgm. dry fat-free solids/ml. mean and *S.E.*	Calculated oxygen uptake on whole-tissue basis (μl./gm./hr.)	Ultimate pH of muscle
Psoas major	1,146 ± 51 (5)	37·0 ± 6·0 (5)	4,770	5·51 ± 0·04 (5)
Sterno-cephalicus	1,554 ± 222 (5)	48·6 ± 6·8 (5)	8,400	5·91 ± 0·08 (5)

* Cytochrome oxidase: Qo_2 max. = μl. oxygen/mgm. dry fat-free enzyme solids/hr. at infinite cytochrome *c* concentration. Experimental conditions of Slater (ref. 9).

intrinsic activity, were greater in the *sternocephalicus* preparations than in those from the *psoas major*. As a result the potential rate of respiration due to cytochrome oxidase in the former was found to be about twice as high as that in *psoas major*, when calculated on a whole-tissue basis. There tends to be an inverse relationship in muscle between respiratory capacity and the magnitude of the initial glycogen store[1,2,11]. (Initial glycogen is an approximation to the *in vivo* resting value. It refers to the quantity at a hypothetical initial *p*H of 7·2, calculated from the *p*H, glycogen content and buffering power of a muscle sample.) Since the *sternocephalicus* has a markedly greater initial store of glycogen than *psoas major*[2], its greater cytochrome oxidase activity is unexpected. This finding does suggest, however, that the glycogen store in a muscle such as ox *sternocephalicus* may serve other purposes than the production of energy by glycolysis or respiration solely from a quantitative aspect. The resistance of part of this glycogen to post-mortem glycolysis, its relative resistance to phosphorylase *b in vitro* and its unusual branching characteristics lead to a similar conclusion.

E. J. Briskey

Animal Husbandry Department,
University of Wisconsin,
Madison.

R. A. Lawrie

Low Temperature Research Station,
Agricultural Research Council and
University of Cambridge.

[1] Lawrie, R. A., *Biochim. Biophys. Acta*, **17**, 282 (1955).
[2] Howard, A., and Lawrie, R. A., *Spec. Rep. Food Invest. Bd.*, London. No. 65 (1957).
[3] Lawrie, R. A., Manners, D. J., and Wright, A., *Biochem. J.*, **73**, 485 (1959).
[4] Fischer, E. H., and Krebs, E. G., *J. Biol. Chem.*, **231**, 65 (1958).
[5] Illingworth, B., and Cori, B., *Biochem. Preparations*, **3**, 1 (1953).
[6] Lawrie, R. A., *J. Physiol.*, **121**, 275 (1953).
[7] Dawes, G. S., Mott, J. C., and Shelley, H. J., *J. Physiol.*, **146**, 516 (1959).
[8] Howard, A., and Lawrie, R. A., *Spec. Rep. Food Invest. Board*, London, No. 63 (1956).
[9] Slater, E. C., *Biochem. J.*, **44**, 305 (1949).
[10] Keilin, D., and Hartree, E. F., *Biochem. J.*, **41**, 503 (1947).
[11] George, J. C., and Naik, R. M., *Nature*, **181**, 709 (1958).

Prostatic Cytochromes

The endogenous respiration of adenomatous human prostatic tissue is known to be low[1]. In the present work the respiratory activity and lactic acid production of normal rat prostate and adenomatous human prostate have been compared.

The rate of oxygen uptake of the human gland was about one-third that of the dorsolateral lobe or the ventral lobe of the rat. It was considerably increased by 10^{-3} *M* succinate and to a lesser extent by 10^{-3} *M* fructose. Except for pyruvate, which had a slight stimulatory effect on the ventral lobe, the oxygen uptake of either lobe of the rat was uninfluenced by 10^{-3} *M* succinate, pyruvate, glucose or fructose (Table 1).

Table 1. Effect of Exogenous Substrates on the Rate of Oxygen Uptake of Prostatic Tissue

Substrate	Oxygen uptake μmoles/gm. dry weight/hr. Tissue: Rat dorsolateral lobe	Rat ventral lobe	Human gland
Nil	80 ± 10 (4)	96 ± 5 (12)	33 ± 7 (8)
Succinate	82 ± 8 (3)	119 (2)	59 ± 5 (5)
Fructose	87 ± 13 (3)	101 ± 21 (3)	53 ± 5 (6)
Pyruvate	84 ± 8 (4)	126 ± 9 (8)	42 ± 6 (6)
Glucose	79 ± 7 (3)	99 ± 12 (3)	32 ± 2 (18)

Slices of tissue were incubated in a calcium-free Krebs–Ringer-phosphate buffer at *p*H 7·4 with 10^{-3} *M* substrate. Equilibrated with air at 37°.
Results are given as means ± *S.E.* with the number of experiments shown in parentheses.

The rate of lactic acid production, which was greater in the human gland than in either lobe of the rat prostate, was increased by added fuctose. All the substrates tested on the rat glands except fructose (which was without effect) slightly raised the rate of lactic acid production. These findings might be due to qualitative or quantitative differences in the cytochrome systems of the tissues (Table 2).

Table 2. Effect of Exogenous Substrates on the Rate of Lactic Acid Production of Prostatic Tissue

Substrate	Lactic acid production μmoles/gm. dry weight/hr. Tissue: Rat dorsolateral lobe	Rat ventral lobe	Human gland
Nil	23 ± 4 (3)	17 ± 1 (5)	90 ± 19 (9)
Succinate	—	24 (2)	65 ± 14 (5)
Fructose	19 (2)	15 (2)	120 ± 10 (6)
Pyruvate	39 ± 6 (4)	27 ± 3 (6)	86 ± 25 (7)
Glucose	31 ± 3 (3)	24 ± 4 (3)	80 ± 10 (18)

Conditions were as shown in Table 1.

Homogenates in phosphate-saline buffer at *p*H 7·4 were prepared from the glandular portions of human adenomatous prostate, and from the separated dorsolateral and ventral lobes of the rat prostate, reduced with sodium dithionite and examined by the Beck–Hartree microspectroscope. Both lobes of the rat gland showed absorption bands with maxima at 550 mμ, 562 mμ and 600 mμ, corresponding to the maxima of cytochromes *c*, *b* and *a*. The bands were also produced by the addition of succinate to cyanide-treated preparations, suggesting enzymic reduction of cytochromes. Homogenates from human glands chemically or enzymically reduced showed strong bands of cytochrome *b* and weaker bands of cytochrome *a*. Cytochrome *c* was not detected with certainty, and, if present, its concentration was considerably less than those of cytochromes *b* and *a*. The cytochrome *b* bands in all preparations disappeared on heating in a boiling water-bath for 5 min.

Fresh normal human prostates have not been available for examination, but the absence or relatively low concentration of cytochrome *c* in the human adenomatous tissue might either be related to the mechanism of adenomatous change, or an adaptive response to hypoxia in the poorly vascularized hypertrophied gland (cf. the decrease in the concentration of cytochrome *c* in diaphragm during hypoxia[2]).

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Leonora Hopkinson
F. L. Jackson

Department of Bacteriology,
Department of Chemical Pathology,
King's College Hospital,
Denmark Hill, London, S.E.5.

[1] Barron, E. S. G., and Huggins, C. J., *Urology*, **55**, 385 (1946).
[2] Duckworth, M. J., *Physiol.*, **152**, 55 *P* (1960).

An Unusual Major Fatty Acid Component in Human Depot Fat

THE technique of gas chromatography has shown that naturally occurring fats and waxes contain saturated and unsaturated fatty acids with both even and odd numbers of carbon atoms. So far, however, the acids with odd numbers of carbon atoms have been reported to occur in only relatively small amounts in natural materials.

During analysis of human depot fat being carried out at this Institute, one specimen was shown to contain 8·7 per cent of a branched chain fatty acid containing thirteen carbon atoms. Other specimens of depot fat we have examined have always contained less than 0·1 per cent of this acid. The analysis showed no other abnormal features. The fat was obtained from the abdominal wall of a male African of the Sukuma tribe, whose approximate age was 60 years, admitted to hospital suffering from a strangulated hernia. The material was treated by the method described by Kingsbury *et al.*[1] and 0·1 μl. of the resulting methyl esters was applied to a 4-ft. column of 20 per cent 'Apiezon *L*' grease on 100–120 mesh 'Celite' held at a temperature of 186° C. Argon was used as the carrier gas and the effluent gas was passed through a Pye ionization detector. The methyl ester of the component emerged from the column with a retention volume of 0·256 relative to methyl palmitate. With these conditions the relative retention volume of *n*-tridecanoic acid was 0·307. The amount of material available was too small to allow the nature of the acid to be determined accurately, but as the peak corresponding to the acid still appeared on the trace after bromination by the method of James and Martin[2] it must be considered to be a saturated acid.

We are unable to explain the presence of the large amount of this acid in this specimen, but since it is most unlikely to occur to any extent in the diet, we suggest that its presence is due to an unusual method of fatty acid synthesis in the body.

D. S. McLAREN
W. W. C. READ

East African Institute for Medical Research,
East Africa High Commission,
P.O. Box 162,
Mwanza, Tanganyika.

[1] Kingsbury, K. J., Paul, S., Crossley, A., and Morgan, D. M., *Biochem. J.*, **78**, 541 (1961).
[2] James, A. T., and Martin, A. J. P., *Biochem. J.*, **63**, 144 (1956).

Blood Glucose-6-Phosphate in Heterozygous Carriers and Patients with Liver Glycogen Disease (Von Gierke's Disease)

Hsia and Kot[1] have reported that children with liver glycogen disease (von Gierke's disease) and their parents, presumably heterozygous for the disease trait, both have high levels of glucose-6-phosphate and fructose-6-phosphate in red blood cells. If correct, this observation would have very important practical as well as theoretical implications. It would greatly simplify the diagnosis and study of this disease and would open the possibility of its control through a eugenic approach. However, Hsia and Kot presented no evidence that their analytical methods were appropriate or that the materials they measured were in fact glucose-6-phosphate and fructose-6-phosphate. Furthermore, the values these authors cited for their normal control population (glucose-6-phosphate, 50–370 mgm. per 100 gm. red blood cells; fructose-6-phosphate, 120–570 mgm. per 100 gm. red blood cells) are much higher than those reported by other investigators[2,3] or expected from theoretical considerations. Accordingly, their findings seemed questionable. We have re-examined this problem with respect to glucose-6-phosphate, using a specific enzymic analytical method. Our results are in sharp disagreement with those of Hsia and Kot.

Samples of whole blood or sedimented erythrocytes were deproteinized with equal volumes of 7 per cent perchloric acid. The filtrates were neutralized with potassium hydroxide and aliquots were incubated with glucose-6-phosphate dehydrogenase and triphosphopyridine nucleotide (TPN). The procedure of Aisenberg[4] was followed, except that 300 μgm. of TPN was used and the total volume of the incubation mixture was reduced to 0·5 ml. The reaction was conducted in microcuvettes. The procedure was applied to standard solutions of glucose-6-phosphate; production of reduced TPN was within 6 per cent of the expected amount. The method was then tested by determining glucose-6-phosphate in whole blood to which different amounts of glucose-6-phosphate had been added. The results are shown in Table 1. There was some loss of added glucose-6-phosphate, presumably due to catabolism during the interval between addition of the material and inactivation of enzymes in the blood. However, recoveries were in a satisfactory range and reproducibility was good, down to concentrations of about 1 mgm./100 ml. Below this level, the procedure proved inaccurate because the increases in optical density during incubation were very small.

Table 1. RECOVERY OF GLUCOSE-6-PHOSPHATE ADDED TO WHOLE BLOOD

Manipulations were carried out at room temperature; time interval before addition of perchloric acid was about 2 min. In each experiment the glucose-6-phosphate concentration was determined in an aliquot of blood to which none had been added and the recovery results were appropriately corrected

Glucose-6-phosphate (mgm./100 ml.) Added	Recovered	Recovery (per cent)
6·4, 6·4, 6·4	5·5, 5·7, 5·6	84, 89, 88
3·2, 3·2, 3·2	3·1, 2·6, 2·9	97, 81, 91
1·6, 1·6	1·4, 1·4	88, 88
0·64, 0·64, 0·64	0·72, 0·53, 0·53	112, 82, 82
0·32, 0·32, 0·32	0·21, 0·34, 0·37	66, 107, 115
0·16, 0·16	0, 0·21	0, 131

Table 2 presents the results obtained on whole blood and/or sedimented erythrocytes in 3 children with von Gierke's disease, 4 parents and 12 other blood relatives of such children, and 11 healthy adults without a family history of the disease. In all cases, the concentrations of glucose-6-phosphate were near the lower limit measurable by the method and much below those reported by Hsia and Kot. There were no major differences among affected children, their parents, other relatives, and normal controls. Because our analytical method was inaccurate at these low concentrations, however, it was not deemed worth

Table 2. GLUCOSE-6-PHOSPHATE IN BLOOD OF CHILDREN WITH VON GIERKE'S DISEASE, RELATIVES OF SUCH CHILDREN, AND NORMAL ADULTS

Relatives examined include 4 parents, 3 grandparents, 4 aunts or uncles and 5 siblings

	Whole blood (mgm./100 ml.)	Red blood cells (mgm./100 ml.)
Affected children		
Case 1	0·4	1·0
Case 2	0·7	—
Case 3	—	0·8
Relatives of affected children, range	< 0·3–0·7 (8 individuals)	< 0·3–0·5 (8 individuals)
Normal controls, range	< 0·3–2·0 (11 individuals)	< 0·3–0·8 (8 individuals)

while to calculate means and standard errors, and our results do not exclude the possibility that small differences between some of these groups may exist.

The recovery data shown in Table 1 indicate that we would not have failed to find the concentrations reported by Hsia and Kot for their normal controls, to say nothing of the very high values these authors reported for affected children and their parents. Our values are roughly of the same order of magnitude as those reported by Bartlett[2], who undertook a painstaking analysis of glycolytic intermediates in human red blood cells, using carefully controlled column chromatographic techniques for the separation of individual compounds and identifying each fraction obtained by appropriate chemical methods. Bartlett obtained the following values in 14 healthy adults: glucose-6-phosphate, 0·08–0·10 μmole per ml. red blood cells (2·1–2·6 mgm. per 100 ml.); fructose-6-phosphate, 0·01–0·02 μmole per ml. red blood cells (0·26–0·52 mgm. per 100 ml.).

Hsia and Kot reported normal erythrocyte concentrations of glucose-6-phosphate and fructose-6-phosphate which are up to six times as high as the normal level of glucose. The concentrations proposed for heterozygous carriers (who have no obvious clinical abnormalities) and for children with von Gierke's disease are ten to fifteen times the normal glucose concentration. These values are far outside the range expected from theoretical considerations or found by other investigators. Since Hsia and Kot offered neither recovery data as a check on their analytical technique nor evidence that the materials measured were indeed glucose-6-phosphate and fructose-6-phosphate, we are forced to conclude that they were measuring something other than these phosphorylated hexoses and that a method for the detection of heterozygous carriers of von Gierke's disease is not yet at hand.

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Joseph E. Sokal
Sylvia Fleissner
Edward J. Sarcione
Charles U. Lowe

Division of Medicine,
Roswell Park Memorial Institute
and the Statler Research Laboratories
of the Children's Hospital,
Buffalo, N.Y.

[1] Hsia, D. Y-Y., and Kot, E. G., *Nature*, **183**, 1331 (1959).
[2] Bartlett, G. R., *J. Biol. Chem.*, **234**, 449 (1959).
[3] Gabrio, B. W., Finch, C. A., and Huennekens, F. M., *Blood*, **11**, 103 (1956).
[4] Aisenberg, A. C., *J. Biol. Chem.*, **234**, 441 (1959).

During the past year, we have attempted to improve the methodology using two different approaches. I would like to summarize the results obtained up to the present time.

In all experiments, equal volumes of 10 per cent trichloroacetic acid were added to whole blood and the precipitate removed by centrifugation. The supernatant was then extracted four times with ether or until neutral, and the ether removed by evaporation.

In the first series of experiments, glucose-6-phosphate was measured directly by incubation with yeast glucose-6-phosphate dehydrogenase (Sigma) and TPN, and observing the conversion of TPN to TPNH in the Beckman spectrophotometer at 340 mμ. Recoveries of glucose-6-phosphate added to normal red cells was somewhat variable, with ranges from 76 to 111 per cent being obtained in successive experiments. Using this technique, the range of values for 6 normal controls was 0·23–0·74 mgm./100 ml. red blood cells (all values have been expressed on the basis that the hexose phosphates are intracellular). In two patients with von Gierke's disease, the values were respectively 0·44 and 0·72 mgm./100 ml. red blood cells. These findings are essentially in agreement with those of Sokal *et al.*

In the next series of experiments, the total hexose monophosphates were determined in the following manner: The evaporated ether extract was passed through 'Dowex 50 × 8 (50–100)' and washed with 25 ml. water. This was neutralized with ammonium hydroxide and put through a 'Dowex 1 × 8 (50–100)' column and washed with 50 ml. water. The resin was eluted with 80 ml. 0·02 *N* hydrochloric acid and the total hexose determined by anthrone. Recovery for glucose-6-phosphate was 101 per cent. Using this technique, the range for normal controls was 1·04–1·77 mgm./100 ml. red blood cells. The values for two mothers were 1·74 and 1·85 mgm./100 ml. red blood cells, and for five children with von Gierke's disease were 1·04, 1·59, 1·87, 2·07, and 2·20 mgm./100 ml. Thus, three of the affected children and one of the mothers had levels in excess of the upper limits of the normal range. These findings are compatible with those previously reported by Wagner *et al.*[1] and from our laboratory.

In reviewing the communication by Sokal *et al.*, one should evaluate critically two points. First, there is considerable doubt as to whether the conversion of TPN to TPNH at 340 mμ can be used as a satisfactory means of measuring glucose-6-phosphate at such low ranges. In particular, I feel that the values by this method tend to run low. Secondly, the authors have failed to take into account the action of phosphatases and other enzymes on glucose-6-phosphate in the erythrocytes of normal individuals[3] and in those with von Gierke's disease. In experiments with normal red cells, we have observed changes of glucose-6-phosphate upon standing at room temperature during a period of 1 hr. The results of Sokal *et al.* in Table 2 would seem to support this. In their experiments, the whole blood was deproteinized within 2 min., while the packed red cells had to be separated by sedimentation or centrifugation involving at least 20–30 min. It can be seen that in case 1, this procedure had little or no effect, since the packed red cell values are approximately twice that in whole blood. On the other hand, in the normal controls the value in the packed red cells, instead of being about twice the range in whole blood, is in fact about half, indicating roughly a four-fold decrease.

We[3] have recently prepared an enzyme system in red cells which hydrolyses glucose-6-phosphate. Assays were carried out using a mixture of 1 ml. of 1 : 1 hæmolysate and 1 ml. 0·04 *M* histidine buffer at 5·0 containing 75 μ*M* of glucose-6-phosphate and 100 μ*M* of ethylene diamine tetraacetate and incubated for 2 hr. at 37° C. The glucose that was liberated was measured by glucose oxidase. Duplicate assays showed variations of less than 5 per cent by this method. The activity in 15 normal controls averaged 17 ± 2·7 mgm. glucose liberated/hr./100 ml. red blood cells, while in 8 patients with glycogen storage disease and their parents, it averaged 14 ± 2·7. The demonstration of an erythrocyte enzyme system which hydrolyses glucose-6-phosphate and a

significant decrease of activity among patients and their parents explains our earlier findings.

DAVID YI-YUNG HSIA
Northwestern University Medical School, Chicago.

[1] Wagner *et al.*, *J. Pediatrics*, **53**, 683 (1957).
[2] Behrendt, H., *Proc. Soc. Exp. Biol. and Med.* **54**, 268 (1943). Tsuboi, K. K., and Hudson, P. B., *Arch. Biochem. and Biophys.*, **43**, 339 (1953).
[3] Inouye, T., and Hsia, D. Y. Y. (to be published).

PHYSIOLOGY

Prolonged Biological Half-Life of Sodium-22 in Patients with Essential Hypertension

In the treatment of hypertension, the effectiveness of sodium depletion by either dietary restriction or natriuretic agents is now fully documented. It is sufficient to say that the response to such treatment led to the hypothesis that hypertensives might suffer from an abnormality in sodium metabolism. The first measurements of the sodium content of individuals with hypertension were done in 1951 by the technique of isotopic dilution using sodium-24 [1]. With the exception of one report these observations have been confirmed repeatedly by ourselves and by others: an increase in sodium was not evident. In the single discordant report [2], some cases of severe hypertension were noted to have an increase in exchangeable sodium, but this was interpreted as being secondary rather than primary to the disease, because it was observed almost exclusively in individuals with advanced hypertension.

In 1953 it was reported that when the average salt-consumption of rats was increased the mean systolic pressure increased linearly [3]. Observations on human beings have shown a similar type of correlation: as the average salt-consumption of a community increases, the prevalence of hypertension increases linearly [4]. Such observations have been interpreted to indicate that chronic excess salt consumption plays a primary part in the ætiology of human hypertension [5]. In view of these considerations, the apparent absence of measurable increase in body sodium in patients with hypertension has been subjected to new scrutiny.

During the past two years we have explored further the sodium metabolism of patients with and without hypertension using sodium-22 (physical half-life, 2·6 years) instead of sodium-24. The results suggest that hypertensives probably do have an abnormality in sodium metabolism.

Fifteen adult patients, none of whom had had symptoms or signs of cardiac or renal failure, were studied. Seven patients had 'essential' hypertension whereas the other 8 had unrelated diseases with normal blood pressure. These investigations were carried out while the patients were in a metabolic ward and were consuming a constant diet which contained about 250 mgm. of sodium chloride by analysis. After the dietary regimen had been established, sodium-22 (usually 2–4 μc.) was given orally and the total body burden of this isotope was recorded every 1–3 days by means of a whole-body counter [6]. Thereafter the patient's daily intake of salt was increased by 2, 5 or 10 gm., at which level it remained for 2–4 weeks without other change in the regimen. Some patients served as subjects for more than one level of salt increment. The addition of this salt resulted in a prompt increase in the rate at which sodium-22 was lost from the body, manifested by a decrease in total body counts. On any given salt intake, the rate of decline in counts proved to be steady and relatively predictable for each patient. At each of the three levels of salt ingestion, the hypertensive individuals, without exception, lost sodium-22 significantly more slowly than did those without hypertension ($P < 0.01$). This is shown in Table 1, where the rate of loss of sodium-22 is described in terms of its 'biological half-life'.

There are at least three possible explanations for the slower excretion of the sodium-22 in hypertensive subjects: (1) The metabolism of sodium-22 differs from that of sodium-23 in hypertensives. This seems most unlikely. (2) Hypertensives absorb less of the supplementary sodium chloride. This was disproved by measurements of the urinary excretion of sodium. (3) Hypertensives have more sodium in the body, that is, they have a larger 'metabolic pool' of sodium. Obviously, the last-named hypothesis merited re-investigation in spite of the repeated evidence to the contrary cited above.

The original calculations of total exchangeable sodium were based on actual weight [1]. However, recent unpublished observations which were made on obese hypertensive females during sharp decline in

Table 1

Patient	Age	Sex	Diagnosis	Weight (kgm.)	Biological half-life of sodium-22 (days) Sodium chloride per day added (gm.) 2	5	10
Hypertensive subjects							
RB	51	M	EHT	83	61 (56, 66)*	28 (26, 30)	13·5 (13, 14)
BC	42	F	EHT	51	65		
HEr	45	M	EHT	62	62		13
AK	56	F	EHT	73	63		
ML	48	M	EHT + P	73			13
PD	58	M	EHT	84	63	26	
NS	55	M	EHT	63	71 (78, 64)	25·5 (26, 25)	13·5 (13, 14)
				Mean	64·2	26·5	13·3
				S.D.	± 3·6	± 1·2	± 0·3
Normotensive subjects							
AE	51	F	Ob	59	48·7 (45, 46, 55)		12 (12, 12)
RG	25	F	Ob	79	41 (40, 42)	20	11
AR	17	F	Ob	55	30	23	11
MW	50	F	ND	64	37 (35, 39)		
MP	52	F	A	47	41	17	9
LL	56	M	Sch	51	39	21	11
CB (coloured)	39	M	Sch	65	45	22	11
EE	60	M	CVA	52	44	20	12
				Mean	40·7	20·5	11·0
				S.D.	± 5·3	± 2·1	± 1·0
				t†	9·27	4·58	± 4·37
				P†	< 0·01	< 0·01	< 0·01

EHT, Essential hypertension; EHT + P, patient also had Parkinson's disease; Ob, obesity; ND, no disease; A, periodic alcoholism, no liver disease; Sch, schizophrenia; CVA, cerebrovascular accident—no known antecedent hypertension.
* More than one value indicates additional periods of study which were not performed sequentially. For such individuals the average of these values was used in calculating mean and standard deviation of the group.
† For hypertensive versus normotensive subjects on equal sodium chloride intakes.

weight had frequently shown negligible loss of exchangeable sodium despite weight losses of 20–50 kgm. This indicated that the fat deposits in such people had a low sodium content. Therefore the calculations based on actual weight might have been misleading if the average hypertensive person had been fatter than the average non-hypertensive subject. Hence, body sodium content was recalculated on the basis of two approximations of 'lean body mass'. This brought into evidence that the hypertensives appeared to have an increase in tissue sodium. One approximation was based on 'desirable' body weight as defined from a recent large actuarial study[7], while in the second, exchangeable sodium was expressed with reference to body potassium. The latter was derived from the amount of naturally occurring potassium-40 present in each patient as determined by a total body radiation counting technique[8].

The evidence seems unequivocal that individuals with essential hypertension have a prolonged biological half-life for sodium 22. Our preliminary calculations suggest that this is the result of hypertensive patients having a large metabolic pool of sodium. These findings lend support to the thesis that sodium is intimately involved in the hypertensive process[4,5].

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Lewis K. Dahl
Malcolm G. Smilay
Lawrence Silver
Sanford C. Spraragen

Medical Research Center,
Brookhaven National Laboratory,
Upton, N.Y.

1 Dole, V. P., Dahl, L. K., Cotzias, G. C., Dziewiatkowski, D. D., and Harris, C., *J. Clin. Invest.*, **30**, 584 (1951).
2 Ross, E. J., *Clin. Sci.*, **15**, 81 (1956).
3 Meneely, G. R., Tucker, R. G., Darby, W. J., and Auerbach, S. D., *J. Exp. Med.*, **98**, 71 (1953).
4 Dahl, L. K., in *Essential Hypertension, An International Symposium*, edit. by Cottier, P., and Bock, K. D., 53 (Springer-Verlag, Heidelberg, 1960).
5 Dahl, L. K., *Nature*, **181**, 989 (1958).
6 Smilay, M. D., Dahl, L. K., Spraragen, S. C., and Silver, L., *J. Lab. Clin. Med.* (in the press).
7 *Statistical Bulletin, Metropolitan Life Ins. Co.*, **40**, 1 (1959).
8 Cohn, S. H., Love, R. A., and Gusmano, E. A., *Science*, **133**, 1362 (1961).

Modification of the Inotropic Activity of Calcium by Potassium

In previous communications from this laboratory[1,2], results have been presented which suggest that the accumulation of calcium at specific sites on or about the membranes defining individual cardiac cells may provide the fundamental basis for the conditions of enhanced contractility recorded during post- and extra-systolic potentiation and throughout the classical staircase[3]. These results confirmed the earlier observations of Niedergerke[4] and support the hypotheses of Sandow[5], of Csapo[6], and of Bianchi and Shanes[7], who maintain that entry of calcium associated with the depolarization stage of activation is involved both in the link between excitation and contraction and in the regulation of the contractile force. Hajdu and Szent-Györgyi[8], however, having observed that the staircase in isolated perfused frog hearts was abolished by reducing the potassium concentration, [K$^+$], of the perfusate suggested that progressive loss of potassium from the myocardium during successive beats of the staircase provided the basis for the increased contractility.

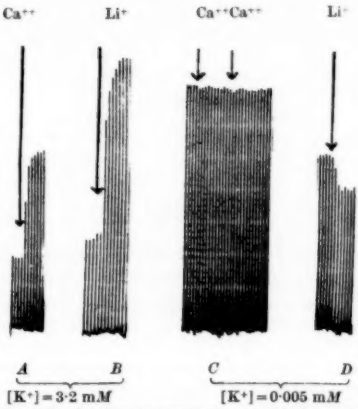

Fig. 1. *A* and *B*. Positive inotropic response recorded following *A*, increase of perfusate [Ca^{++}] from 0·65 to 2·6 m*M* when [K$^+$] was 3·2 m*M* (Ca^{++} added at arrow); *B*, replacement of half the perfusate Na$^+$ by Li$^+$ (at arrow) when the perfusate [K$^+$] was 3·2 m*M*

C and *D*. Change in contractile force recorded in same preparation as used in *A* and *B* following *C*, increase of perfusate [Ca^{++}] from 0·65 to 2·6 m*M* when the perfusate [K$^+$] was reduced to 0·005 m*M*; *D*, replacement of half the perfusate Na$^+$ by Li$^+$ when the perfusate [K$^+$] was 0·005 m*M*

A possible link between the hypothesis involving changed [K$^+$] formulated by Hajdu and Szent-Györgyi, and that involving the accumulation of calcium as postulated by Niedergerke and others, has recently been noted in a series of experiments in which the action of calcium on the contractile behaviour of isolated toad ventricles perfused with Ringer's solution containing varying [K$^+$] was studied.

In these experiments ventricles removed from summer, non-hibernating toads of the species *Bufo marinus* were stimulated at a constant rate of 6 beats/min. using supra-maximal rectangular pulses delivered from a 'Tektronix' square-wave generator. Isotonic contractions detected by the photo-electric method previously described[9] were recorded on a direct-writing recorder and the ventricles initially perfused for a standard 60 min. equilibration period with Ringer's solution containing 3·2 m*M* K$^+$ and 0·65 m*M* Ca^{++}. Following this preliminary perfusion, addition of Ca^{++} (as $CaCl_2$) to give a final [Ca^{++}] of 2·6 m*M* resulted in an immediate well-defined positive inotropic response (see Fig. 1*A*). Such an increase in contractile force would be anticipated from the classical experiments of Ringer[10]. Similarly, replacement of half the perfusate Na$^+$ by an isotonically equivalent amount of Li$^+$ (as LiCl) evoked an immediate positive inotropic response (Fig. 1*B*).

Reduction of the perfusate [K$^+$] from the original level of 3·2 m*M* to 0·005 m*M*, profoundly modified these positive inotropic responses recorded in the presence of 3·2 m*M* K$^+$ when the perfusate [Ca^{++}] was increased or the [Na$^+$] decreased. Thus when the perfusate [K$^+$] was reduced to 0·005 m*M*, increasing the [Ca^{++}] from the original 0·65 m*M* to 2·6 m*M* either failed to alter the contractile force or in some preparations slightly decreased it (see Fig. 1*C*). In the presence of this low [K$^+$], replacement of half the perfusate Na$^+$ by Li$^+$ without any attendant change in tonicity reduced the amplitude of contractions (see Fig. 1 *D*).

The negative inotropic response which was recorded when the [Ca^{++}] of the perfusate was raised from a

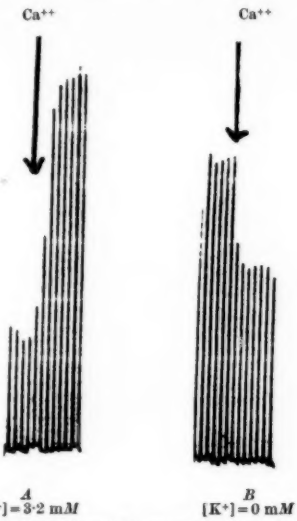

Fig. 2. Changes in contractile force recorded in a single preparation following the addition (at arrow) of Ca^{++} to give a final concentration of 2·6 m*M* when the perfusate contained *A*, 3·2 m*M* K^+; *B*, K^+ deleted

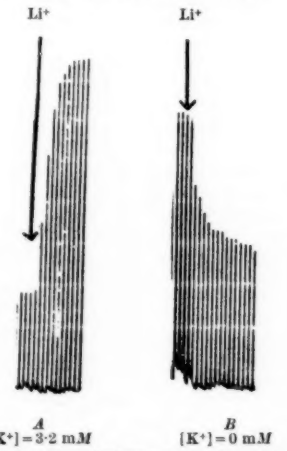

Fig. 3. Changes in contractile force recorded from a single preparation following replacement (at arrow) of half the perfusate Na^+ by an isotonically equivalent amount of Li^+ when *A*, perfusate contained 3·2 m*M* K^+; *B*, K^+ deleted from perfusate

preliminary level of 0·65 m*M* to 2·6 m*M* or when the $[Na^+]$ was halved was further enhanced by the use of a K^+-free perfusate. A typical result is shown in Fig. 2*A* and 2*B*, where the positive inotropic response recorded, following the above increase in perfusate $[Ca^{++}]$ in the presence of 3·2 m*M* K^+, is compared with that recorded from the same preparation following a similar increase in $[Ca^{++}]$ in the absence of any perfusate K^+. Similarly, replacement of half the perfusate Na^+ by Li^+ depressed the contractile activity when K^+-free perfusate was used (see Fig. 3*A* and 3*B*). In these particular experiments using the K^+-free solution it was necessary to replace repeatedly the K^+-free perfusate throughout the preliminary perfusion so that K^+-loss from the myocardium associated with successive contractions would not ultimately result in an appreciable perfusate $[K^+]$.

From these results it seems possible that the failure of Hajdu and Szent-Györgi to record the classical staircase in the absence of K^+ may reflect the need for the presence of a critical perfusate $[K^+]$ for Ca^{++} to exert a positive inotropic effect on cardiac muscle. The findings of Hajdu and Szent-Györgyi, therefore, do not preclude the involvement of calcium in the staircase and other conditions of enhanced cardiac contractility.

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WINIFRED G. NAYLER

Baker Medical Research Institute,
Melbourne.

[1] Nayler, W. G., *J. Gen. Physiol.*, **44**, 393 (1960).
[2] Nayler, W. G., *J. Gen. Physiol.* (in the press).
[3] Bowditch, H. P., *Bericht über die Verhandlungen der KGL (Akademie der Wissenschaften zu Leipzig)*, **23**, 562 (1871).
[4] Niedergerke, R., *J. Physiol.*, **134**, 569 (1956).
[5] Sandow, A., *Yale J. Biol. Med.*, **25**, 176 (1952).
[6] Csapo, A., *Ann. N.Y. Acad. Sci.*, **81**, 453 (1960).
[7] Bianchi, C. P., and Shanes, H. M., *J. Gen. Physiol.*, **42**, 803 (1959).
[8] Hajdu, S., and Szent-Györgyi, A., *Amer. J. Physiol.*, **168**, 159 (1952).
[9] Nayler, W. G., and McCulloch, M., *Austral. J. Exp. Biol.*, **38**, 117 (1960).
[10] Ringer, S., *J. Physiol.*, **4**, 29 (1883).

Free Amino-Acids in Rachitic Epiphyseal Cartilage of Albino Rat and the Influence of Vitamin D

IT was suggested by Dikshit and Patwardhan[1] that administration of vitamin D imparted an enhanced calcifying capacity to rachitic bone and cartilage. Histochemical work by Ramalingaswami *et al.*[2] on cartilage in rickets and during healing under the influence of vitamin D, revealed an alteration in the staining characteristics of the cartilage matrix on the basis of which they suggested that vitamin D influenced the glycoprotein of the rachitic cartilage matrix. Dikshit[3] observed a decrease in the hexosamine content of the rachitic cartilage during healing induced by the administration of vitamin D. It was felt desirable, therefore, to investigate whether vitamin D influenced the protein metabolism of the cartilage. The effect of vitamin D on the free amino-acids of rachitic cartilage was studied as a preliminary to such investigations.

Young albino rats, 4 weeks old, were fed a low-phosphorus rachitogenic diet[4] for 14 days and then divided into 3 groups taking litter and sex-distribution into account. Each rat in group 3 received a single dose of 4,000 I.U. vitamin D orally on the fourteenth day and those in group 2 on the sixteenth day. Rats in group 1 received no vitamin D and served as rachitic controls. On the eighteenth day, 4–12 rats from each group were killed and their tibial epiphyseal cartilages removed, pooled according to groups, and weighed. The weighed cartilage was extracted exhaustively with 75 per cent ethanol, the collected extracts were evaporated and dried, and the residue was extracted again with 95 per cent ethanol. The residue from the second extract after evaporation was dissolved in 10 per cent isopropanol.

Using phenol/isopropanol/water (70 : 5 : 25) in presence of 0·3 per cent ammonia as the developing solvent, serine, glycine, threonine, glutamic acid and glutamine were separated from the mixture of free amino-acids contained in the isopropanol solution of cartilage extracts. Circular paper chromatographic procedure was used as described by Giri *et al.*[5] for

Table 1. Effect of Vitamin D on the Free Amino-acids of Rachitic Rat Cartilage

Amino-acid		Rachitic controls (no vitamin D) After 48 hr. I	Treated rats (4,000 I.U. vitamin D/rat) After 48 hr. II	After 96 hr. III
Glutamic acid	Mean	222	194*	222†
	S.E.	± 4·1	± 2·5	± 7·2
Serine	Mean	14·3	18·8*	24·2*†
	S.E.	± 1·45	± 0·39	± 0·51
Glycine	Mean	68	80	99*
	S.E.	± 2·1	± 4·7	± 6·5
Threonine	Mean	17·0	10·8*	15·5†
	S.E.	± 1·49	± 0·78	± 1·12
Glutamine	Mean	124	104*	122†
	S.E.	± 1·8	± 2·3	± 4·2

Values denote μgm. of amino-acid/100 mgm. fresh tissue.
* Difference significant from group I below 5 per cent level.
† Difference significant from group II below 5 per cent level.
Each value is a mean of 3 observations; each observation was on cartilage pooled from 4–12 rats.

quantitative determination of these amino-acids. Duplicate values at three concentration-levels were obtained and only those in which there was good agreement within 5 per cent selected.

Results shown in Table 1 revealed that administration of vitamin D affected the concentration of some free amino-acids in the rachitic cartilage. While serine and glycine showed a gradual increase during healing, glutamic acid, glutamine and threonine showed an initial decrease by 48 hr. followed by a rise to the pre-treatment level at 96 hr. These different trends observed preclude the possibility that other simultaneous changes in the cartilage, for example, changes in the concentration of calcium and phosphorus[6], are the cause of the observed changes in the concentration of the free amino-acids.

Thus there appears unmistakable evidence that protein metabolism in the rachitic epiphyseal cartilage altered under the influence of vitamin D.

G. V. G. Krishna Rao

Nutrition Research Laboratories,
Indian Council of Medical Research,
Tarnaka, Hyderabad-7.

[1] Dikshit, P. K., and Patwardhan, V. N., *Ind. J. Med. Sci.*, **6**, 107 (1952).
[2] Ramalingaswami, V., Sriramachari, S., Dikshit, P. K., Tulpule, P. G., and Patwardhan, V. N., *Ind. J. Med. Sci.*, **8**, 509 (1954).
[3] Dikshit, P. K., *Nature*, **183**, 334 (1959).
[4] Dikshit, P. K., and Patwardhan, V. N., *Ind. J. Med. Res.*, **35**, 91 (1947).
[5] Giri, K. V., and Rao, N. A. N., *Nature*, **169**, 923 (1952); *J. Ind. Inst. Sci.*, **34**, 95 (1952). Giri, K. V., Krishna Murthy, K., and Venkita Subramanian, T. A., *Curr. Sci. (India)*, **21**, 11 (1952).
[6] Dikshit, P. K., and Sriramachari, S., *Ind. J. Med. Res.*, **49**, 117 (1961).

Failure of the Purified Growth Hormone to restore the Size of the Kidneys in Hypophysectomized Rats

In previous work the effect of hypophysectomy on the size of the remaining kidney after unilateral nephrectomy was investigated in dogs and rats[1]. After hypophysectomy rats, unlike dogs, lose weight or fail to grow normally. It is, therefore, difficult to assess whether the failure of the remaining kidney to hypertrophy is a non-specific effect or the result of a deficiency of (a) particular hormone(s). Bearing this in mind, it seemed necessary to study the effect of growth hormone.

Young male rats, weighing between 35 and 120 gm., were hypophysectomized by the parapharyngeal approach. Of the hypophysectomized animals, one group (six rats) received growth hormone while another group (ten) was untreated. After the operation the animals were given drinking water containing glucose and, in addition to the ordinary rat diet, brown bread, milk and glucose were given.

Purified growth hormone was dissolved in faintly alkaline isotonic solution of sodium chloride and was injected beneath the skin twice a day with a 7-hr. interval between the two injections, in doses indicated in Table 1.

Table 1. Effect of Substituting Growth Hormone on the Weight of the Kidney of Hypophysectomized Rats

Body-weight (gm.) Initial	Body-weight (gm.) Final	Weight of both kidneys (mgm.)	Remarks
46	102	676	Injected with 2 mgm. growth hormone for 20 days
54	182	831	
35	86	823	
84	124	843	Injected with 4 mgm. growth hormone for 20 days
115	192	1,350	
72	148	1,035	

Grant from N.H.I. (United States); N.I.H.-*GH-B2* (bovine growth hormone); growth potency, 1·53 × U.S.P. reference standard; contamination LH, 0·01; follicle stimulating hormone, 0·009 U.S.P.; prolactin, 0·33 I.U.; adrenocorticotrophic hormone, 0·25 U.S.P.; thyroid stimulating hormone, 0·009 U.S.P.

The animals were killed with ether on the twenty-first day after hypophysectomy. The sella turcica was carefully examined for any remnants. If the pituitary was completely removed, the sella turcica was seen to be covered with a smooth glistening layer of the meninges, beneath which a central depression in the bone, representing the site of the drill hole, was seen.

From Table 1 it would appear that the growth hormone was effective in promoting growth in the hypophysectomized rats and the rate of growth in the treated animals was quite comparable with that of normal animals. The adrenals and testes, however, were found to be lighter in weight than in the normal controls. In contrast to these findings, the kidneys of hypophysectomized rats receiving growth hormone were found to be the same size as those from the untreated hypophysectomized animals. This conclusion was reached as a result of expressing kidney size as the ratio of kidney weight to body-weight. Statistical analysis of the results showed that there was no significant difference in kidney size between the hypophysectomized group and the group which was treated with the hormone, but that there was a significant difference between these two groups and the control normal group (Table 2). It is interesting that the stomach weight and gastric mucosa were found restored completely to normal in the hypophysectomized animals and those treated with growth hormone (Crean, G. P., personal communication). It can be seen that injections of the purified growth hormone can maintain the growth of the hypophysectomized rat, but are unable to prevent the relative shrinkage of the kidney that occurs after this operation. This is contrary to results with the crude extract of the adenohypophysis[1].

Depression of kidney functions and blood-flow are known to occur after hypophysectomy[2,3]. Unlike adenohypophysial crude extract, gonadotrophic hor-

Table 2. Effect of Growth Hormone on Kidneys of Hypophysectomized Male Rats

No. of rats	Condition of rat	Kidney weight in mgm./body-weight in gm. Mean (± *S.D.*)	Statistical significance
13	Normal	9·533 (± 2·14)	*P* 0·01
10	Hypophysectomized	7·461 (+ 0·84)	None *P* 0·01
6	Hypophysectomized, growth hormone	6·931 (± 1·59)	

mone, thyroid stimulating hormone and adrenocorticotrophic hormone, when given individually, are unable to restore kidney function completely to normal.

From these results it seems unlikely that an explanation in terms of hæmodynamic changes[4] of the failure of the kidney to grow is correct, for the kidney remained small in hypophysectomized rats even when the animals grew at a normal or slightly greater than normal rate. It is more likely that there is a deficiency of a pituitary hormone without which the kidney cannot well maintain its size and growth in the hypophysectomized animals. It would appear, from this work, that growth hormone is unlikely to be the only deficient hormone.

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T. Astarabadi

Department of Physiology and Endocrinology,
Research Unit (Medical Research Council),
University of Edinburgh.

[1] Astarabadi, T., and Essex, H. E., *Amer. J. Phys.*, **173**, 326 (1953).
[2] Thompson, K. W., and Galser, D. W., *Yale J. Biol. and Med.*, **4**, 677 (1932).
[3] De Rodo, R. C., *et al.*, *Fed. Proc.*, **10**, 33 (1951).
[4] Earl, D. P., *et al.*, *Amer. J. Phys.*, **173**, 188 (1953).

Effect of Urea Ingestion on Parotid Salivation in Sheep

Urea is the most common source of non-protein nitrogen used for the partial replacement of protein in the diet of ruminants. In association with readily available carbohydrate such as molasses, urea is used to supplement the low-quality roughage diets which are often consumed by ruminants in Africa, the United States and Australia.

This form of supplementation results in an increase in the rate of cellulose digestion in the rumen[1]. Food intakes are increased by approximately 30–40 per cent, live-weight losses are decreased, and wool production is increased[2–5].

Balch and Johnson[6] found a correlation between the fluidity of the rumen contents and the rate of cellulose digestion. It is therefore important to know if the volume of saliva secreted is altered when urea and molasses are added to a roughage diet.

The volumes of parotid saliva secreted by two Merino wethers were measured by means of permanent unilateral parotid fistulæ. Each sheep weighed approximately 40 kgm. and they were kept in electrolyte balance by allowing them to drink their own saliva.

The basic diet consisted of oaten straw containing 2 per cent crude protein. The supplement of urea (25 gm.), molasses (200 gm.) and water (200 gm.) was intimately mixed with straw (600 gm.) to make up the daily ration. This supplement raised the nitrogen content of the straw to the equivalent of 11 per cent crude protein.

One sheep received straw only, while the other received straw plus the supplement. After two weeks of recordings, the diets were reversed. The unsupplemented straw was offered without stint and the daily intake per sheep was approximately 500 gm. The intake of straw when fed with the supplement was restricted by offering only 600 gm. of straw plus the supplement. On every occasion this ration was completely consumed.

The volumes of saliva secreted did not alter significantly with the addition of the supplement. The average volumes for each animal were 2,160 and 2,560 ml. without the supplement, and 2,250 and 2,380 ml., respectively, with the supplement.

The mean total nitrogen of the saliva from each animal rose from 2·9 and 4·2 mgm. nitrogen/100 ml. to 12·1 and 13·3 mgm. nitrogen/100 ml. respectively, when the supplement was added to the straw.

It is concluded that the increased rate of passage of food through the alimentary tract, and the increased rate of cellulose digestion in the rumen, which are brought about by urea feeding, are not associated with quantitative changes in the pattern of parotid salivation. In the present work the urea supplement produced an increase of 20 per cent in the consumption of straw, but this was not accompanied by any increase in parotid salivation.

A reasonable estimate for the total volume of saliva secreted by each sheep would be 10 l. a day[7]. Also, it has been shown that the nitrogen content of this total salivary secretion, excluding mucoprotein, is similar to that of parotid saliva[8]. Since the total nitrogen content of parotid saliva was raised by 10 mgm./100 ml. on feeding urea, the increase in saliva nitrogen represents an overall increase in nitrogen economy to the sheep of only about 1 gm. nitrogen a day.

Therefore, the influence of urea supplementation on the parotid secretion does not seem to be particularly important from either the qualitative or quantitative point of view.

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A. D. Wilson

School of Agriculture,
University of Melbourne.

[1] Coombe, J. B., and Tribe, D. E., *Proc. Austral. Soc. Anim. Prod.*, **3**, 83 (1960).
[2] Clark, R., and Quin, J. I., *Onderstepoort J. Vet. Res.*, **25**, 93 (1951).
[3] Beames, R. M., *Queensland J. Agric. Sci.*, **16**, 223 (1959).
[4] Coombe, J. B., *J. Austral. Inst. Agric. Sci.*, **25**, 299 (1959).
[5] Williams, N. M., Pierce, G. R., Delaney, M., and Tribe, D. E., *Emp. J. Exp. Agric.*, **27**, 107 (1959).
[6] Balch, C. C., and Johnson, V. W., *Brit. J. Nutr.*, **4**, 389 (1950).
[7] Kay, R. N. B., *J. Physiol.*, **150**, 515 (1960).
[8] Phillipson, A. T., and Mangan, J. L., *N.Z. J. Agric. Res.*, **2**, 990 (1959).

HISTOCHEMISTRY

Relationship between Tissue Eosinophils and Peroxidase Activity

Four different peroxidase enzymes have been found in mammalian organisms: lactoperoxidase, myeloperoxidase, hepatic tryptophan peroxidase and labile peroxidase of the thyroid gland. There is insufficient knowledge of the physiological significance of these enzymes for animal organisms. This applies especially to the myeloperoxidase found in the granules of leucocytes[1,2].

It has been shown histochemically that the peroxidase activity possessed by eosinophils in the rat differs from other granulocytes[2]. This enzyme is very resistant in eosinophils to the effect of, say, formalin, ethyl and methyl alcohol and other agents of similar type and it is therefore possible to demonstrate it specifically by histochemical methods. It can, however, be asked whether this enzyme is a myeloperoxidase at all.

The highest peroxidase concentrations of the extramedullary organs of the rat have been established in

the small intestine, uterus, large intestine, spleen, lungs and stomach[3]. It is in these same organs that the relatively highest extra-medullary eosinophil counts have also been established in the rat[2]. It has also been observed that both the peroxidase concentration[4] and the number of eosinophils[2] increase clearly in the rat uterus under the influence of œstrogen stimulation. It is thus possible that the high peroxidase content of the organs mentioned derives from eosinophilic granulocytes.

The observation that the peroxidase enzyme in rat uterus is in its behaviour like lactoperoxidase[4] —and not like myeloperoxidase—agrees well with the histochemical differences existing between eosinophils and other granulocytes.

The views cited are supported by the fact that physiological, intense eosinophilia appears in lactating lactiferous gland[5].

The high peroxidase concentration established *in vitro* in the gastrointestinal tract, uterus, spleen and lungs is probably caused by the eosinophil granulocytes of tissues. The enzyme contained in the eosinophils resembles lactoperoxidase.

Tapio Rytömaa
Harald Teir

Department of Pathology,
Section II, University of Helsinki.

[1] Agner, K., *Acta Chem. Scand.*, **12**, 89 (1958).
[2] Rytömaa, T., *Acta Path. Microbiol. Scand.*, **50**, Supp., 140 (1960).
[3] Neufeld, H. A., Levay, A. N., Lucas, F. V., Martin, A. P., and Stotz, E., *J. Biol. Chem.*, **233**, 209 (1958).
[4] Martin, A. P., Neufeld, H. A., Lucas, F. V., and Stotz, E., *J. Biol. Chem.*, **233**, 206 (1958).
[5] Fawcett, D., in *Histology*, ed. R. Greep (J. and A. Churchill, London, 1954).

PATHOLOGY

Acid Extract of Leucocytes and *Staphylococcus pyogenes*

Several workers[1–6] have demonstrated recently the inhibitory action of acid extracts of leucocytes towards microbes.

Amano[7] *et al.* found a cell-free acid extract of guinea pig leucocytes to be lethal to coagulase-negative *Staphylococci*, and to be slightly inhibitory to coagulase-positive *Staphylococci*. This active principle they call leucozyme *A* and show that although lysozyme is present it is not the agent responsible for the destruction of the *Staphylococci*.

Hirsch[8] has also reported on the destructive activity of an acid extract (phagocytin) of leucocytes obtained from several species. This extract is prepared free of lysozyme. I have confirmed both these observations using *Staphylococcus albus* and *aureus* and extracts made from guinea pig, rabbit and human leucocytes. My work has been extended to compare the activity of human and rabbit phagocytin towards *Staphylococcus pyogenes* obtained from a variety of lesions which exhibit qualitative and quantitative differences in toxin production; I also compared the action of an extract made from leucocytes obtained from patients with acute osteomyelitis with that from healthy donors.

It seemed reasonable to expect that *Staphylococcus pyogenes* which have survived phagocytosis and proliferate in the bone marrow would show a greater resistance to phagocytin. The results, shown in Table 2, do not support this. No significant difference is found in the action of either of the extracts towards *Staphylococci*.

Table 1. Reduction in Staphylococcal Colony Counts by Phagocytin

No. of strains staphylococcoid *pyogenes*		Human phagocytin extract. Col. count reduced to: 10 or less per cu. cm.	10/20 per cu. cm.	Rabbit phagocytin extract. Reduction of from 140 to 10 or less per cu. cm.
Nasal	4	4	0	4
Boils	3	1	2	3
Osteomyelitis	30	26	4	30
Miscellaneous	23	16	7	23
Total	60	47	13	60

Average count for untreated controls, 130–140 colonies per cu. cm.

Table 2

No. of strains		Normal human white blood cells extract. Col. count 10 or less	10–20	Osteomyelitic white blood cells extract 10 or less	10–20
Nasal	2	2		2	
Osteomyelitis	8	6	2	6	2
Total	10	8	2	8	2

Average count for untreated controls, 130–140 colonies per cu. cm.

The extracts have been made by Hirsch's methods, incubated for 2 hr. with a 4-hr. shaken culture of the test organism in broth standardized to give a reading of 1·0 on an EEL colorimeter using an orange filter. After incubation this standard extract and suspension is finally diluted to 1/50,000 and pour plates made with 5 ml. agar in standard 2-in. Petri dishes. Controls are set up using distilled water instead of the extract. The colonies are counted per cubic centimetre; for the controls the average count is between 130 and 140 per c.c.

In view of the Japanese report that their acid extract was more inhibitory towards coagulase-negative *Staphylococci*, an attempt was made to see whether a partially purified coagulase preparation gave any protection. No difference was noted when this was used. The *p*H at which tests were made fell to *p*H 5·6–6·0. (The coagulase was prepared by the late Dr. Edward Duthie of Southampton.)

Coagulase, α toxin, staphylokinase, leucocidin (some) and hyaluronidase production of the *Staphylococci* were noted, and there is no correlation between the toxigenicity of the *Staphylococci* tested and their susceptibility to phagocytin, or the nature of the lesion from which the organism was originally isolated; the two strains showing greater resistance to the osteomyelitic extract were different from those two relatively resistant to the normal extract. Neither is there any apparent relationship between phagocytin susceptibility and the ability to provoke antibody response in the patient to the various antigens of the coccus. The morphology of the *Staphylococci* surviving incubation with extracts is normal, as might be expected from the appearance of the colonies.

A. G. Towers

Department of Pathology,
Institute of Orthopaedics,
Royal National Orthopaedic Hospital,
Brockley Hill, Stanmore, Middlesex.

[1] Rogers, D., and Melly, M. A., *J. Exp. Med.*, **111**, 533 (1960).
[2] Kapral, F. A., and Shayegani, M. Gh., *J. Exp. Med.*, **110**, 123 (1959).
[3] Van Heyningen, W. E., *J. Gen. Microbiol.*, **5**, 758 (1951).
[4] Myrvik, Q. N., and Weiser, R. S., *J. Immunol.*, **74**, 9 (1955).
[5] Fishman, M., and Silverman, M. S., *J. Exp. Med.*, **105**, 521 (1957).
[6] Morse, S. I., *Nature*, **186**, 102 (1960).
[7] Amano, T., *et al.*, *Med. J. Osaka Univ.*, **7**, 233 (1956).
[8] Hirsch, J. G., *J. Exp. Med.*, **111**, 323 (1960).

Lathyrogenic Activity *in vitro*

REASONS have already been given for the view that the basic aortic lesion in experimental lathyrism is mainly a failure of formation of elastic tissue[1]. An attempt has since been made to investigate, *in vitro*, the effect of various lathyrogenic substances on the production of elastic fibres.

Explants were taken from the ventricles of chick embryo heart (the great vessels being carefully removed so that the only elastic tissue in the initial explants was the small amount present in the coronary vessels) and were cultured by a modification of the method described by Bloom[2] using Waymouth's medium[3] with 10 per cent calf serum for 10–12 days at 39° C. as the fluid phase. Whether the explants were grown in fibrin clot or directly on glass, in stoppered glass tubes or in Petri dishes in an atmosphere with 5 per cent carbon dioxide did not affect the results. Some additional results obtained from experiments with mouse embryo hearts do not differ markedly from those with chick embryo hearts and are not reported in detail here.

A preliminary experiment showed that fibres similar to those described by Bloom[2] first appeared after 7 days incubation, and were well formed after 10 days. Subsequent cultures were therefore examined after this time. The fibres stain with Weigert's method for elastica, with orcein, and with Gomori's aldehyde fuchsin, providing strong presumptive evidence that they are indeed elastica; further investigations are being made into this matter. The fibres usually run parallel to each other to form a circle or arc of a circle around the explant at the junction of the original tissue and the monolayer extending from it. They appear to have formed immediately under the surface layer of cells at the farthest point from the glass. In a few cases, particularly in small explants, the fibres form a disorganized network, or may show some radial orientation. In the presence of lathyrogenic substances these fibres were not found.

For assay of lathyrogenic activity each experiment comprised multiple cultures and graded concentrations of test substances. Only those experiments in which one or more control cultures produced elastica were considered. In successful cultures 40 out of 51 (78 per cent) of the controls produced elastica. When β-aminopropionitrile hydrochloride—known to be lathyrogenic *in vivo*—was added to the cultures it was found that a concentration of 2 mgm./ml. killed the majority of the explants, and in the few cases where the cells survived elastic fibres were never formed. With lower concentrations down to 80 μgm./ml., although the explants were healthy as frequently as in the control cultures, elastic fibres were never formed. With still lower concentrations the percentages of healthy cultures producing elastic fibres were as follows: 16 μgm./ml., 10 per cent; 3·2 μgm./ml., 12·5 per cent; 0·64 μgm./ml., 66 per cent. Two other compounds active *in vivo*, β-mercaptoethylamine[4] and semicarbazide[5], inhibited the formation of elastic fibres at concentrations of 3·4 μgm./ml. and 0·66 μgm./ml. respectively. Sodium propionate and *n*-propylamine, both inactive *in vivo*, were also inactive *in vitro* at concentrations of up to 364 μgm./ml. and 0·16 per cent respectively; higher concentrations caused cell death. Assuming uniform distribution of lathyrogenic substances throughout animal tissue, the doses of β-aminopropionitrile hydrochloride, β-mercaptoethylamine and semicarbazide which inhibit the formation of elastica *in vitro* correspond to the concentrations calculated as being present in the tissues of mice treated with these compounds and showing rupture of the aorta. The results demonstrate that lathyrogenic substances act at a cellular level, and the parallelism between the *in vivo* and *in vitro* findings supports the original hypothesis that the aortic rupture in experimental lathyrism is secondary to a failure of formation of normal elastic tissue.

H. MORAG MCCALLUM

Department of Pathology,

JOHN PAUL

Department of Biochemistry,
The University,
Glasgow.

[1] McCallum, H. Morag, *Nature*, **182**, 1169 (1958).
[2] Bloom, W., *Arch. Exp. Zellforsch.*, **9**, 6 (1929).
[3] Waymouth, Charity, *J. Nat. Cancer Inst.*, **22**, 1003 (1959).
[4] Dasler, W., and Milliser, R. B., *Proc. Soc. Exp. Biol. Med.*, **98**, 759 (1958).
[5] Dasler, W., *Proc. Soc. Exp. Biol. Med.*, **97**, 112 (1958).

An Early Test for Possible Skin Carcinogens

IN previous papers[1–4] it has been shown that it is possible by a tetrazolium reduction method to estimate changes in the rate of formazan deposition in the epidermis of hairless mice after different skin irritants.

A characteristic reaction was observed after the application of some carcinogenic substances and after local irradiation with X-rays. The topical application of carcinogens produced a short initial rise in formazan deposition, followed by a depression of longer duration. An initial decrease in formazan deposition was observed after the application of some non-carcinogenic compounds. It was suggested that an initial rise in formazan deposition might be specific for skin irritants with carcinogenic potency.

Examining the validity of this hypothesis, a blind-test with 20 different compounds was performed. The substances were dissolved in benzene and the different samples were indicated by *A*, *B*, *C*, . . . etc.

Hairless mice, 8 males and 8 females for each test substance, were given 0·005 ml. of the solution as a single, topical application to a circumscribed area of the skin. The formazan deposition per mgm. dry epidermis per hour measured on the first and the second day after the application was recorded. It was postulated that mean relative values of 1·100 and above should indicate a compound with skin carcinogenic potency.

The results of the blind test are given in Table 1. The values are arranged in order from the lowest to the highest. To the right is presented the list of answers, including an indication of the carcinogenic potency of the substances. In this communication only those substances are listed as complete carcinogens which each alone are known to produce epidermoid carcinomas in mouse skin after topical application.

It appears that there is a correlation between such complete carcinogenic activity and initial rise in formazan deposition. The above-mentioned hypothesis is thus given some further support and the method may probably be used as an early test for possible skin carcinogens. It must, however, be kept in mind that correlation never proves specificity.

Table 1

Blind test			List of answers	
Compound	Mean	S.D. of the mean	Compound	Skin carcinogenic potency
H	0·682	0·07	1,2 benzanthracene	–
L	0·754	0·11	Pyrene	–
K	0·770	0·07	*d*-Limonene	–
F	0·825	0·12	1 stearyl 2,3 polyethylene monoglyceride	–
D	0·827	0·12	Naphthalene	–
J	0·838	0·09	3 methyl 1,2 benzanthracene	–
S	0·848	0·06	Oil of turpentine	–
G	0·880	0·13	Fluoranthene	–
T	0·881	0·09	Cholesterol	–
I	0·897	0·09	Phenanthrene	–
E	0·908	0·07	Ethyl carbamate	–
B	0·909	0·08	Anthracene	–
Q	0·970	0·05	1,2,6,7 dibenzanthracene	–
P	1·010	0·08	1,2,3,4 dibenzanthracene	–
O	1·307	0·12	4 nitroquinoline N oxide	+
C	1·337	0·11	Croton oil	+
N	1·405	0·12	9,10 dimethyl 1,2 benzanthracene	+
A	1·408	0·08	3,4 benzpyrene	+
R	1·410	0·09	3 methylcholanthrene	+
M	1·634	0·12	1,2,5,6 dibenzanthracene	+

The substance *P* (1,2,3,4 dibenzanthracene), for which a borderline value (1·010) was observed, is the substance that represents an exception in the interesting observations of Heidelberger[5] concerning binding of carcinogens to skin proteins.

There is no general agreement as to what tetrazolium really measures. It acts as an electron acceptor and is thereby reduced to formazan. The amount of formazan deposited per mgm. dry epidermis per hour may probably be considered as an estimate of the activity of the energy-generating processes in the cell, mainly the mitochondrial enzyme systems. Increased values may mean some injury to the mitochondria, for example increased permeability of the mitochondrial membrane.

Olav Hilmar Iversen

Institutt for Generell og Eksperimentell Patologi, Universitetet i Oslo.

[1] Iversen, O. H., *Acta Path. et Microbiol. Scand.*, **47**, 216 (1959).
[2] Iversen, O. H., *Acta Path. et Microbiol. Scand.*, **50**, 291 (1960).
[3] Iversen, O. H., *Acta Path. et Microbiol. Scand.* (in the press).
[4] Iversen, O. H., and Devik, F., *Intern. J. Rad. Biol.* (in the press).
[5] Heidelberger, Ch., Ciba Foundation Symp. Carcinogenesis, London, 1959.

PALÆONTOLOGY

A Mammalian Fauna from the Kimmeridgian of Portugal

A MAMMALIAN fauna, consisting of 14 specimens, has been collected from the small Guimarota coal pit, near Leira. The age has been determined as Kimmeridgian by means of the ostracods and characeans. The pit is still in operation and it seems possible to obtain a good fauna, qualitatively as well as quantitatively. A typical coal swamp fauna has been encountered. Lamellibranchs, gastropods, and ostracods are frequently found; the holostean genus *Lepidotus* is well represented by scales. Carapace fragments of turtles are less frequently found. Crocodiles predominate numerically, with many shed teeth and scutes. Many lizard jaws have been collected, as well as a few shed teeth of dinosaurs.

The fauna contains the earliest known multituberculates (two good jaws without molars and one skull without teeth) and the oldest pantotherian upper dentition. The latter has a tooth formula of P^4/M^5 (P^{2-4} have been lost, P^4 prior to fossilization). There is also a good pantotherian lower jaw which has a tooth formula of $I\ /C_1/P_4/M_6$. All the molars are unbasined, the trigonid being open lingually. The talonid is covered by the paraconid of the tooth behind; it is not worn by the opposing lingual cusp of the upper molars. The lower molars have their longitudinal axis oblique to the axis of the jaw.

The molars are shorter than those of *Amphitherium* and the talonid is much smaller than in the latter tooth. The metaconid almost equals the protoconid in height and is higher than the paraconid. This is a feature which is found also in the dryolestid genera *Dryolestes*, *Laolestes*, *Phascolestes* and *Perasalax* as well as in the paurodont genera *Archaeotrigon* and *Tathiodon*. The tooth formula of the present jaw is now diagnostic in respect to differentiation between Amphitheriidae and Dryolestidae, but it excludes paurodont relationship.

The right lower, M_3, and right upper, M^3, of the pantothere are shown in Figs. 1 and 2. There is no suggestion that these upper and lower jaws belong to the same genus. The tooth shown in Fig. 2 is typical as regards number of cusps on all teeth. M^{1-3} are longer than broad, M^4 and M^5 are broader than long. The tooth is similar in many respects to *Herpetairus arcuatus* from the Morrison formation. Both have the same number of cusps of equal relative height. A large basin is formed by five of six cusps, two buccally, two posteriorly and one, the highest, lingually. The sixth cusp, at the anterobuccal corner, stands outside

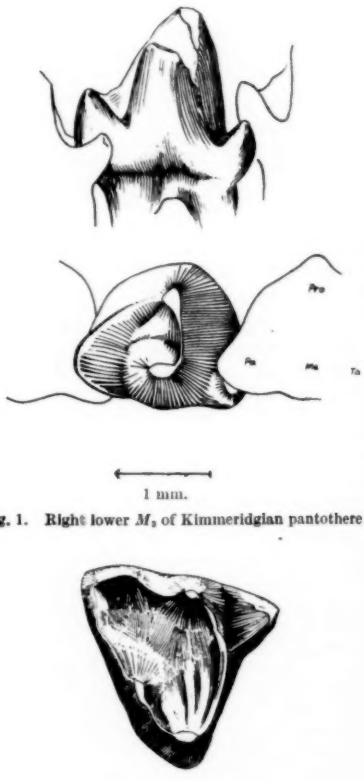


Fig. 1. Right lower M_3 of Kimmeridgian pantothere

Fig. 2. Right upper M^3 of Kimmeridgian pantothere

the triangular basin and dorsal to it. The function of this cusp is to buttress the posterobuccal corner of the tooth in front; similarly the talonid cusp buttresses the paroconid. The second highest cusp, as in *Melanodon*, *Herpetairus*, *Amblotherium* and *Kurtodon*, stands at the anterobuccal corner of the basin.

Primitive features of the lower jaw and maxilla, seeing that they are older than Purbeckian, are: (1) in the jaw there is an open, not a closed trigonid (the Purbeckian paurodontids with an open trigonid are similar to the Bathonian *Amphitherium* in this respect); it is agreed that in *Amphitherium*, the earliest known pantothere, the open trigonid is a primitive character which the paurodonts retain; (2) it has been postulated that in pantotherian upper molars of pre-Purbeckian age the length would equal the width in all teeth. In the Kimmeridgian upper dentition now available, M^{1-3} are longer than broad, whereas in the Purbeckian only M^1 has such dimensions.

It has been customary to discuss the origin of the Pantotheria whenever a new mammalian fauna in the Mesozoic is discovered. Such procedure is inevitable, taking into consideration the few faunas known and the interest that the Pantotheria arouse as ancestors of the placentals and marsupials. I do not agree with a derivation of Pantotheria from Rhætic symmetrodonts, as Butler[1], Kermack and Mussett[2], and Patterson[3] do. I consider a derivation from Rhætic triconodonts more likely. The new maxillary dentition does not provide any new item of symmetrodont affinity. The second largest cusp (Butler's buccal cusp, Patterson's stylocone) stands right in the middle of the buccal side as in Purbeckian teeth, forming a tricuspid front with the cusp at the anterobuccal corner (Butler's anterior cusp, Patterson's parastyle) and the cusp at the postero-buccal corner (Butler's posterior cusp, Patterson's "metastyle"). Patterson's cusp on the anterior crest of some Purbeckian dryolestids, said to indicate symmetrodont affinity, is not present on any of the molars of the Kimmeridgian maxilla. The central buccal cusp has in the new maxilla the same relative height as in the Purbeckian teeth; while if it were to be a homologue of the anterobuccal cusp of the Symmetrodonta, one would expect it to be less high.

The morphological break between upper premolar and molar is considerable in the dryolestid upper dentition. Only the aberrant *Docodon* has a P^4 which is so similar to the M^1 that the nature of the paracone and protocone in the molars of this genus has never been debated. The paracone stands in the middle of the buccal row of cusps, flanked by two smaller cusps; the protocone is a lingual neomorph, added to an original triconodont pattern.

I believe that *Docodon* and all other Pantotheria have—as Simpson[4] originally claimed—the same cusp pattern and the same origin. A history of all cusps of the Albian therian from Texas, of the Purbeckian maxilla, of the new Kimmeridgian maxilla and the Rhætic triconodonts or symmetrodonts can be written irrespective of whether one begins with a triconodont or a symmetrodont. With a triconodont at the base a much simpler history of all dental elements concerned is possible. Moreover, this would solve the *Docodon* paradox, namely, that one genus which is patently neither triconodont nor symmetrodont has a different origin from all (other) Pantotheria.

As regards jaws as well as teeth, the Purbeckian *Docodon* was a living fossil, giving a hint of what happened to the still unknown triconodonts which, in the Upper Liassic, embarked on the road to the Pantotheria.

Walter G. Kühne

Geologisch-palaeontologisches Institut
der Freien Universität Berlin,
Berlin-Dahlem,
Altensteinstrasse 34*a*.

[1] Butler, P. M., *Proc. Zool. Soc., Lond.*, **109**, Parts 3 and 4, 329 (1939).
[2] Kermack, K. A., and Mussett, F., *Discovery*, 144 (April 1959).
[3] Patterson, B., *Fieldiana*, **13**, No. 1 (1956).
[4] Simpson, G. G., *American Mesozoic Mammalia* (1929).

Chitinozoa from the Ordovician Nod Glas Formation of Merioneth

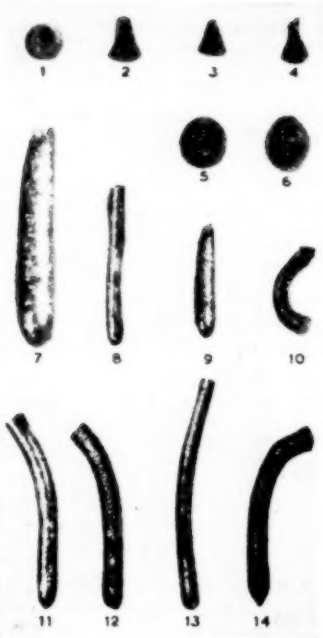
Chitinozoa[1] are microscopic, flask-like fossils of problematical affinities. They are generally black, structureless and opaque, being composed of 'pseudochitin', and ranging from 0·03 mm. to 1·5 mm. in length. They are bilaterally symmetrical and may be club-like, rod-like, or globular in form, the test being thin, hollow and open at one end. The surface of the test is usually smooth, but is sometimes tuberculate. Spines may be present and aggregates of up to six individuals have been recorded[2].

The zoological affinities of the Chitinozoa have been discussed by Collinson and Schwalb[3], who conclude that they represent an extinct order of rhizopod protozoans.

Chitinozoa are known from strata ranging from Ordovician to Devonian in age from Germany, France, Czechoslovakia, the United States, Canada and Brazil. The only previous record of Chitinozoa from Britain is that of Lewis[3], who described two species of *Conochitina* (C. cf. *C. filifera* Eisenack and *C. cactacea* Eisenack) in thin sections of the Upper Carodocian "Phosphate band" of Pen-y-garnedd, Montgomeryshire.

The present specimens were collected from crags on the face of the Graig Tynant (M.R. SH/898218), about two miles north of Llanmawddy, Merionethshire[4]. They occur in limestone nodules 15 ft. above the base of the Nod Glas Formation[4], and were extracted by digestion of the limestone in acetic acid.

The Nod Glas chitinozoa fall into three well-defined morphological groups. The most common are elongated tubular forms, with the oral end open and the aboral end roundly closed. Most of these (for example, Fig. 8) are straight and agree broadly with Eisenack's description of *Rhabdochitina magna* Eisenack, although they show rather more variation in the form of the distal end than is recorded by Eisenack. Numerous other specimens show, however, that there is a complete gradation between such forms and those with a gently or strongly curved axis (Figs. 11–14) and also between forms which have a uniform diameter along their entire length (that is, 'tubular' forms) and those in which the aboral diameter is rather larger than the oral (that is, 'club-shaped' forms). Such forms as the latter have been referred to the genus *Conochitina* Eisenack, but the present specimens suggest that it is difficult to separate these from 'typical' members of the genus *Rhabdochitina*. The axial curvature does not seem sufficient to exclude most of these forms from the species *R. magna*. A precedent for the evaluation of such variation as infra-specific is provided by Eisenack[5],



Figs. 1–14. Chitinozoa from the Ordovician Nod Glas Formation of Merioneth. (× *c.* 44)

1–4, *Illichitina* cf. *I. campanulaeformis* (Eisenack): 1, oral view of specimen, showing cross-section of cylindrical neck and basal outline of chamber; 2–4, oblique lateral views of specimens showing general form of chamber

5–6, *Lagenochitina*? sp.: Lateral views showing general form. 7 and 9, Club-shaped forms of *Rhabdochitina*. (*Conochitina* of some authors)

8, 11–14, *Rhabdochitina* spp.: lateral views of specimens showing variation in general form of chamber

10, fragmentary specimen. Gen. et sp. indet.

who included various forms showing comparable variation within the species *Rhabdochitina taenia* Eisenack.

The second group of chitinozoa are bell-shaped forms (Figs. 1–4) which closely resemble the species *Illichitina campanulaeformis* (Eisenack). The base is often perforated, and they appear to show morphological variation which partly bridges the gap between that species and *I. crotalum* Collinson and Schwalb.

The third group are globular forms (Figs. 5 and 6), which may represent the genus *Lagenochitina* Eisenack. Some have an invaginated opening, but all appear to lack the tubular neck which is characteristic of most members of the genus.

I am most grateful to Prof. Harry B. Whittington, of Harvard University, who accompanied me in the field, and to Mrs. Shirley Osborn, who has given me considerable help in the extraction of specimens.

F. H. T. Rhodes

Geology Department,
University College,
Swansea.

[1] Eisenack, A., *Palaeontologische Z.*, **12**, 74, pls. 1–5 (1931).
[2] Collinson, C., and Schwalb, H., North American Paleozoic Chitinozoa. *Illinois State Geol. Surv.*, 7 (1955).
[3] Lewis, H. P., *Ann. and Mag. Nat. Hist.*, **5**, 1, pls. 1–4 (1940).
[4] Pugh, W. J., *Quart. J. Geol. Soc. Lond.*, **85**, 242 (1931).
[5] Eisenack, A., *Palaeontologische Z.*, **14**, 257, pls. 11–12 (1932).

BIOLOGY

Growth-stimulating Activity of *Trans*-Caffeic Acid isolated from *Coleus rhenaltianus*

In our previous work[1] on the abscission of debladed petioles of *Coleus rhenaltianus*, we met with an auxin that was not an indole derivative, in ether extracts from the leaves.

On paper chromatograms of ethanolic leaf and stem extracts, developed with isopropanol/water (5 : 1 v/v), we now find two blue fluorescent spots (R_F 0·84 and 0·79), the eluates from which both enhance the elongation growth of *Avena* coleoptile sections (Table 1).

Table 1. Growth-rate of 3 mm. *Avena* Coleoptile Sections in a Solution of 1 per cent Glucose and the Eluate from a Chromatogram (Spots at R_F 0·79 and 0·84) of an Ethanolic Leaf and Stem Extract of *Coleus*. Growth recorded after 24 hr. Number of Sections between Brackets

Increase in length (eye-piece micrometer units)			
Spots at R_F 0·79		Spots at R_F 0·84	
Control	Diluted eluate	Control	Diluted eluate
16·8 ± 1·59 (15)	26·8 ± 1·50 (14)	13·0 ± 0·72 (15)	19·4 ± 0·76 (17)
16·6 ± 1·34 (13)	26·2 ± 1·73 (14)	8·4 ± 0·89 (10)	20·4 ± 1·04 (10)
8·4 ± 0·89 (10)	23·9 ± 1·86 (9)	5·0 ± 0·45 (10)	10·8 ± 0·58 (10)

The eluates show practically the same ultra-violet absorption spectrum as the auxin found earlier in ether extracts of leaves. The substance at R_F 0·84 was identified by us as *trans*-caffeic acid. Most probably the substance at R_F 0·79 is a closely related one, since it gives several identical colour reactions and, after treatment with diluted sulphuric acid, is transformed into *trans*-caffeic acid, with R_F 0·84. It is neither chlorogenic nor *cis*-caffeic acid. Both compounds are precipitated with neutral lead acetate; after removal of the lead from the yellow precipitate, growth activity in the coleoptile section test is unimpaired.

Generally the cinnamic acid derivatives caffeic and ferulic acid are considered to be growth inhibitors[2]. Other reports mention a synergistic action of these substances with the growth regulators indole-3- and α-naphthyl-acetic acid[3,4]. Caffeic acid alone is thought not to exert any true auxin activity. We find, however, that commercial *trans*-caffeic acid, at a concentration of about 1 μgm./ml. or less, greatly enhances the elongation growth of coleoptile sections (Table 2), about as much as does indole-3-acetic acid in these circumstances.

Table 2. Growth-rate of 2 mm. *Avena* Coleoptile Sections in Solutions of *trans*-Caffeic Acid and Indole-3-acetic Acid in Distilled Water. Both Substances were added 6 hr. after cutting the Sections. Growth has been recorded after 18 hr. Number of Sections between Brackets

Increase in length (eye-piece micrometer units)					
trans-Caffeic acid			Indole-3-acetic acid		
Control	1 μgm./ml.	0·5 μgm./ml.	Control	1 μgm./ml.	0·5 μgm./ml.
3·8 ± 0·45 (16)	5·7 ± 0·35 (21)	4·3 ± 0·34 (18)	3·0 ± 0·48 (17)	5·0 ± 0·82 (17)	4·8 ± 0·60 (10)
1·2 ± 0·28 (14)	5·0 ± 0·34 (14)	3·7 ± 0·27 (18)	1·5 ± 0·54 (10)	3·0 ± 0·59 (10)	3·2 ± 0·46 (11)
2·2 ± 0·42 (15)	5·6 ± 0·34 (15)	4·4 ± 0·44 (13)	2·4 ± 0·34 (14)	5·5 ± 0·54 (14)	4·6 ± 0·58 (14)

The rather general occurrence[5] of caffeic acid in plant tissues thus suggests that this substance may be a very important natural growth regulator, not less important than indole-3-acetic acid.

Of both stereo-isomeric cinnamic acids, only the *cis*-isomer is physiologically active in growth[6]. Our own experiments have confirmed once more the inactivity of *trans*-cinnamic acid. The *cis* configuration has been supposed to be generally important in growth regulation. It is believed, moreover[7], that the introduction into the lipophilic aromatic nucleus of

hydrophilic substituents results in the formation of inactive or weakly active compounds. Taking into account these structural requirements, we should not at all expect auxin activity in *trans*-caffeic acid, which is 3′,4′-dihydroxy cinnamic acid. The extreme difference in growth activity between *trans*-cinnamic and *trans*-caffeic acid on the contrary seems to indicate a special importance of the ortho-diphenol configuration in growth regulation.

Our results stress the importance of a thorough study of the physiological function of cinnamic acid derivatives in growth and metabolism.

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J. C. VENDRIG
K. BUFFEL

Laboratorium voor Plantenphysiologie,
Carnoy Instituut,
Louvain.

[1] Vendrig, J. C., *Wentia*, **3**, 1 (1960).
[2] Akkerman, A. M., and Veldstra, H., *Rec. Trav. Chim. Pays Bas*, **66**, 411 (1947).
[3] Reinders-Gouwentak, C. A., and Smeets, L., *Proc. Kon. Akad. Wet Amsterdam, C*, **56**, 354 (1947).
[4] Hemberg, T., *Physiol. Plant.*, **4**, 437 (1951).
[5] Tomaszewski, M., *Acad. Pol. Sci., Ser. Biol.*, **8**, 61 (1960).
[6] Veldstra, H., *Enzymologia*, **11**, 97, 137 (1944).
[7] Veldstra, H., *Ann. Rev. Plant Physiol.*, **4**, 151 (1953).

High Root-Pressures in Palms

DESPITE some impressive demonstrations of root-pressure[1–3], 'pumping' or positive pressure is generally thought to be of little importance for the ascent of sap in trees. This is probably true for dicotyledons, but in ten species of palms at Calcutta I was able to detect and measure positive root-pressures throughout the year, sometimes enough to lift sap to heights exceeding those of the trees.

A palm has rather uniform adventitious roots growing from the bole, or stem base. The number at bearing stage varies from a few hundred to more than 13,000 as in *Cocos nucifera*. Such a root, generally about 0·7 cm. in diameter, is cut about a metre from the bole. The proximal end, attached to the tree, is connected by a long rubber tube to a glass tube of 3 mm. bore dipped in water, and connexions sealed with paraffin. The distal end, severed from the tree, is connected by a short rubber tube either to a vertical glass capillary tube or a mercury manometer.

The maximum suction, or negative pressure, is usually reached within 10 hr., and may persist for as long as four months. Its value fluctuates rapidly with atmospheric changes, as would be expected if transpiration were an important cause of it. The positive pressure usually starts developing after about 24 hr., and reaches a maximum after 10–20 days, the time varying between species and between roots of the same tree. After a day or two it begins to decline, and disappears after about two months. There are slight diurnal fluctuations, the pressure being higher by day. Table 1 gives data on 51 palm and 2 banana (*Musa sapientum*) trees. The roots of *Phoenix sylvestris* are not suitable for the operation described, and the low values obtained are probably due to leakage. In seven of the eleven species, the positive pressure exceeds the negative pressure considerably. It often exceeds the plant's needs. Thus a seedling of *Borassus flabellifer*, a little under a metre high, had a root which developed a pressure of 5·24 m. of sap. Again, in *Musa sapientum*, the only monocotyledon other than a palm which I studied, and which behaves just like the palms, the suckers die before reaching a height of 4 m.

In order to demonstrate that my results are due to a physiological property of palms, and not to differences of method, I used just the same methods on

Table 1

Species of trees studied	Number	No. of roots connected for		No. of roots showing		Maximum height (cm.) of water column	
		Suction pressure	Pumping pressure	Suction	Pumping	Sucked	Pumped
Cocos nucifera	11*	20	39	20	34	20·1	1,250
Borassus flabellifer	10	29	36	27	24	51·0	524
Areca catechu	6	25	25	23	19	27·5	550
Livistonia chinensis	6	16	24	16	15	42·0	100
Phoenix sylvestris	5	13	20	12	1	18·2	34
Ptychosperma macarthuri	4	19	19	17	13	34·3	650
Oreodoxa regia	3	5	12	4	6	25·9	650
Chrysalidocarpus lutescens	3	9	15	8	7	39·9	310
Livistonia rotundifolia	2†	5	5	4	4	24·0	40
Caryota urens	1‡	3	3	3	1	62·0	70
Total for the 10 species	51	144	198	134	124	62·0§	1,250§
Musa sapientum	2	11	11	10	9	25·1	670

* Two of these palms were studied at Kayangulam, Kerala State.
† Connexions damaged due to heavy rains at an early stage.
‡ The palm was at its last stage of flowering.
§ Denotes the maximum for all the palms studied.

Table 2

Species of trees studied	Number	No. of roots connected for		No. of roots showing		Maximum height (cm.) of water column	
		Suction pressure	Pumping pressure	Suction	Pumping	Sucked	Pumped
Dalbergia sissoo	1	6	6	6	nil	54·5	0
Polyalthia longifolia	1	5	5	5	nil	59·0	0
Sterculia urens	1	5	5	4	nil	27·6	0
Psidium guajava	1	5	5	5	nil	61·2	0
Ficus religiosa	1	4	4	4	nil	27·5	0
Total	5	25	25	24	nil		
Cycas circinalis	1	7	7	5	nil	35·7	0

five dicotyledonous trees and one gymnosperm. The results are given in Table 2, and require no comment.

It seems likely that monocotyledonous plants, having no secondary growth, behave very differently from dicotyledons. As M. H. Zimmermann (private communication) says: "because most monocots do not form new vascular tissue in the lower part of the trunk as dicots do, a cohesion mechanism alone would run into difficulties". The method is clearly important as others[1-6] have failed to demonstrate root pressure in palms.

A few simple experiments suggest that the observed root pressures suffice to drive sap up the stem about as easily as up a capillary tube. The distal part of a cut root of a fruited banana plant was seen to develop pressure. The plant was decapitated at 2·25 m. above ground-level, and the cut root reconnected with a rubber tube. Other agencies such as transpiration are probably less important than root pressure.

T. A. Davis

Indian Statistical Institute,
Calcutta-35,
India.

[1] Hales, S., *Vegetable Staticks* (1727) [*Some Apostles of Physiology* (Waterlow and Sons, 1902)].
[2] Merwin, H. E., and Lyon, H., *Bot. Gaz.*, **48**, 442 (1909).
[3] White, P. R., *Amer. J. Bot.*, **25**, 223 (1938).
[4] Bose, J. C., *The Physiology of Ascent of Sap* (Longmans, Green and Co., London, 1923).
[5] Bose, J. C., *The Motor Mechanism of Plants* (Longmans, Green and Co., London, 1928).
[6] Molisch, H., *Bot. Z.* (1902) (quoted by Bose).

Probable Mechanism of Resistance of Varieties of Cocoa to Black Pod Disease caused by *Phytophthora palmivora* (Butl.) Butl.

Isolates of *Phytophthora palmivora* showed different degrees of pathogenic activity (measured by rate of spread of lesions at 25° C.) when wound-inoculated into surface-sterilized pods of particular varieties of cocoa (*Theobroma cacao*, L.). One isolate, *WA* 8, failed to attack any variety. Isolates also showed varied rates of spread in different varieties of cocoa. The variety *SCA* 6 was consistently more resistant than the variety *ICS* 1. Holliday[1] has shown that the variety *SCA* 6 is more resistant to artificial inoculation of intact pods with a zoospore suspension than is the variety *ICS* 1.

Rotted cocoa pod tissue showed no evidence of pectolytic enzyme activity when tested by Brown's maceration method[2], and by the polygalacturonase agar plate method[3]. The same tests, however, showed that pectolytic enzymes were produced by *Phytophthora palmivora* on autoclaved potato blocks.

Aqueous extracts of host tissue, prepared by homogenization of tissue in distilled water (during which browning occurred), when added to pectolytic enzymes of *P. palmivora* from invaded autoclaved potato blocks caused inhibition of the pectolytic enzyme activity. The inactivating ability of such extracts was removed by pre-treatment with 0·5 per cent aqueous solution of gelatin. If the homogenization was carried out in 0·1 per cent aqueous sodium dithionite solution browning did not occur and the extracts did not cause inhibition of pectolytic enzyme activity. These results suggested that the inhibition was due to high molecular weight tannins produced by polymerization of the products of oxidation of host polyphenols by polyphenol oxidase[4-7].

Tests of macerating activity of pectolytic enzymes of *P. palmivora* on cocoa pod tissue showed that tissue disks were more resistant to maceration when allowed to brown after cutting than if browning were prevented by immersion in a 0·1 per cent sodium dithionite solution. Moreover, disks of the more resistant variety *SCA* 6 browned more rapidly than those of the susceptible variety *ICS* 1 and showed correspondingly greater resistance to maceration.

If the resistance of cocoa varieties to rotting by isolate *WA* 8 were due to the inactivation of pectolytic enzymes of the fungus by action of the host polyphenol oxidase system, then reduction of the activity of the latter system by low oxygen tension would be expected to break down this resistance. Some of these effects have been demonstrated. Cocoa pods of variety *IMC* 67 were wound-inoculated with the avirulent isolate *WA* 8, placed at 60, 21 and 2 per cent oxygen tension and incubated at 25° C. Lesions were produced at 2 per cent oxygen tension but were very light brown in colour, whereas at oxygen tensions of 21 and 60 per cent no lesions were produced, the only reaction being a narrow dark margin around the point of inoculation.

Pods inoculated with isolate *WA* 8 and incubated at 15° C. produced lesions, whereas no attack occurred at 25° C. or 30° C. Low temperature also might be expected to reduce the polyphenol oxidase activity and this reduction in host resistance would explain the better growth of the fungus in the host tissue at low temperatures than at temperatures nearer the optimum for growth of the fungus on agar culture.

A virulent isolate produced lesions at 60, 21 and 4 per cent oxygen tensions; size of lesions increased from an average diameter of 2·1 cm. at 60 per cent, to 3·3 cm. at 21 per cent and 4·5 cm. at 4 per cent after three days incubation at 30° C. The lesions varied from dark brown at 60 per cent, to brown at 21 per cent and lighter brown at 4 per cent, indicating a correlation between rate of attack and browning of host tissues.

Tests of growth-rates of *P. palmivora* at various temperatures and oxygen tensions showed that the results could not have been solely due to direct effects on the growth-rate of the parasite. Other host factors would be affected by alteration of oxygen tension and temperature, but an alteration of the balance between the pectolytic enzyme systems of the parasite and the polyphenol oxidase system of the host offers the simplest explanation of promotion of attack by low oxygen tension and low temperature.

If resistance is correlated with polyphenol oxidase activity of cocoa pod tissue it may be possible to select varieties with high activity of the polyphenol oxidase system, and if, as would be expected, these show some degree of resistance, to use them in breeding resistant varieties.

It is hoped that a fuller account of this work will be submitted for publication shortly.

John A. Spence

Department of Botany,
University of Bristol.

[1] Holliday, P., *Rep. Fifth Conf. Inter-American Tech. Cocoa Comm.* (1954).
[2] Brown, W., *Ann. Bot.*, **29**, 313 (1915).
[3] Dingle, J., Reid, W. W., and Solomons, G. L., *J. Sci. Food Agric.*, **4**, 149 (1953).
[4] Griffiths, L. A., *Nature*, **180**, 1373 (1957).
[5] Griffiths, L. A., *Biochem. J.*, **70**, 120 (1958).
[6] Byrde, R. J. W., *J. Hort. Sci.*, **32**, 227 (1957).
[7] Byrde, R. J. W., *Phenolics in Plants in Health and Disease* (Pergamon Press, 1960).

Canadian Salmon caught off Greenland

On October 10, 1960, an Atlantic salmon 71 cm. total length, weighing 3·7 kgm. (8·2 lb.), and carrying Fisheries Research Board of Canada tag No. 1,616, was caught in the sea off Tassiussaq (65° 06′ N., 52° 08′ W.), near Napossok to the south of Sukkertoppen, Greenland. The fish was tagged on May 22, 1959, in the estuary of the Miramichi River near Chatham, New Brunswick (47° 04′ N., 65° 28′ W.), as a smolt 17·5 cm. in total length. It was one of 3,500 smolts tagged there in May and June, 1959, with tags patterned after those used by Dr. Börje Carlin[1] in Sweden, but had printed laminated plastic pendants supplied by a commercial firm.

Scales from the recaptured fish show three years of river life typical of Miramichi River smolts, followed by a first sea year of moderate growth and a relatively wide winter band. The second sea year includes a period of fast growth followed by a summer check, then a period of fast growth continuing to the time of capture in October.

This recapture represents the longest migration yet recorded, about 1,500 miles, for Canadian Atlantic salmon the river of origin of which is known through fin-clipping or tagging. Many fin-clipped salmon from Quebec, New Brunswick, and Nova Scotia rivers have been recaptured, however, in sea fisheries as far away as Newfoundland and Labrador. The recapture also lends some support to the suggestion[2] that salmon from both sides of the North Atlantic share common feeding grounds. The first direct evidence of Scottish salmon reaching Greenland waters was provided by the recapture in October 1956 near Sukkertoppen, Greenland, of a fish tagged as a kelt in November 1955 at Loch na Croic, Ross-shire[3].

We are indebted to Dr. Jørgen Nielsen, of the Ministeriet for Grønland, for details of this recapture accompanied by a scale sample, and to Dr. P. F. Elson, of the Biological Station, St. Andrews, New Brunswick, for reading the scales.

C. J. Kerswill
M. H. A. Keenleyside

Fisheries Research Board of Canada,
Biological Station,
St. Andrews, New Brunswick.

[1] Carlin, Börje, *Institute of Freshwater Research, Drottningholm, Report* No. 36 (1955).
[2] Menzies, W. J. M., *The Stock of Salmon. Its Migrations, Preservation and Improvement*, 96 (London, 1949).
[3] Menzies, W. J. M., and Shearer, W. M., *Nature*, **179**, 790 (1957).

Penetration of Spines from a Marine Diatom into the Gill Tissue of Lingcod (*Ophiodon elongatus*)

Fishermen from the Porlier Pass area of Vancouver Island were experiencing unusually high mortalities among lingcod (*Ophiodon elongatus*), which according to common practice were being held in surface live-wells prior to the weekly slaughtering and transport to market. Examination of dead and dying fish showed only that all specimens had discoloured gills with considerable detritus embedded in the gill mucus (Fig. 1). Much of this detritus was composed of *Chaetoceros convolutus*, one of a common group of marine plankton diatoms[1], and their siliceous spines (Fig. 2). The spines or setæ with their apically directed barbs are shown in Fig. 3. These findings suggested the possibility that the setæ might bring about tissue damage and be a direct or indirect cause

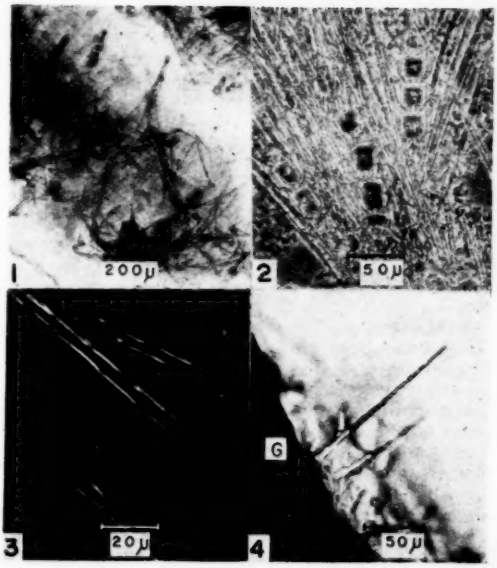

Fig. 1. *Chaetoceros* cells and setæ on surface of lingcod gill filaments. Specimen had died in live-well within 8 hr. of sampling

Fig. 2. Phase contrast view of *Chaetoceros* sp. and setæ on gill mucus. Same specimen as in Fig. 1

Fig. 3. Dark field picture of *Chaetoceros* setæ showing apically directed barbs

Fig. 4. Setæ embedded in gill of dead lingcod which had been exposed for 24 hr. at 11° C. to 1–5 × 10^5 *Chaetoceros* cells/l. *G* indicates gill tissue

of death. Gill tissue is particularly vulnerable to injury from chemical and biotic agents because of its intimate contact with the external environment and because of its delicate structure. It was therefore decided to expose apparently healthy lingcod (18 in.) to 3–4 times the natural concentration (that is, 1–5 × 10^5 cells/l.) of *Chaetoceros* while the fish were held in aquaria. Control fish were kept in natural sea water.

Only one of the three treated fish died, from undetermined causes, and although the final results were inconclusive, it is of considerable interest that setæ were found embedded in the gill tissue of the dead fish (Fig. 4). It was established that the spines had actually penetrated the tissue and were not merely trapped in the mucus. The incidence and extent of the penetration were not determined, but four embedded setæ were seen in an area of a few square millimetres. The only comparable record appears to be that of Wood and Yasutake[2], who found that ingested 'hairs' from the lepidopterous larva *Halisidota argentata* penetrated the stomach and visceral organs of fingerling salmon by working their way inwards and thus causing extensive damage to the tissues. The insect hairs, like the algal setæ, have apically directed barbs and therefore both shafts must enter butt first for maximum penetration. This direction of entry into lingcod gill tissue is shown in Fig. 4. Setæ which are entrapped on or embedded in the gills might cause death by capillary hæmorrhage, by anoxia from the stimulation of heavy mucus secretion or by debilitation of the tissue allowing microbial invasion.

It is not yet possible to say whether this penetration is only a laboratory oddity or whether it is also a significant natural phenomenon, but the subject

appears to warrant further investigation by marine biologists.

I am very grateful to Dr. T. Parsons of the Pacific Oceanographic Group for his valuable advice and assistance.

Gordon R. Bell

Fisheries Research Board of Canada,
Biological Station, Nanaimo, B.C.

[1] Cupp, E. E., *Bull. Scripps Inst. Oceanogr. Univ. Calif.*, **5**, 100 (1943).
[2] Wood, E. M., and Yasutake, W. T., *J. Parasit.*, **42**, 544 (1956).

An Intra-Pericardial Hepatic Intrusion in the Common Dogfish

Miss Barbara Stevens, of Brooklands Technical College, has been kind enough to send me a dogfish (*Scyliorhinus canicula*) with an intra-pericardial hepatic intrusion. Only the anterior end of the dogfish is available; but the length from the tip of the snout to the anterior insertion of the pectoral fin is 9 cm., and the anterior ends of the testes present a mature appearance. Thus it may be inferred that the dogfish had practically reached full size with this abnormality when it was caught.

The septum transversum is perforated on the right side only, and the hepatic intrusion is remarkable for its extent (Fig. 1). On the right side the liver extends through the pericardial cavity for a distance of 18 mm., almost reaching the anterior end of the conus arteriosus. Laterally, the liver extends 23 mm., reaching across almost the entire width of the pericardial cavity at its posterior end. The heart appears to be normal, except that the ventricle is displaced slightly forward and to the left.

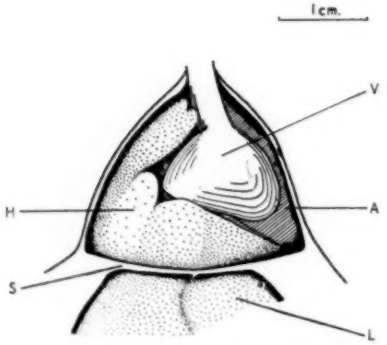

Fig. 1. Hepatic intrusion in the pericardial cavity of a dogfish. *A*, Auricle; *H*, hepatic intrusion; *L*, liver; *S*, septum transversum; *V*, ventricle

The volume of the intra-pericardial intrusion was estimated by making a 'Plasticine' replica and measuring the volume of this by displacement. This was found to be 2·7 c.c. Similarly, the volume of the heart was found to be 2·2 c.c. Thus the hepatic intrusion occupied rather more of the pericardial cavity than the heart. The volumes of the hearts of a small number of dogfish under class dissection were also estimated by making 'Plasticine' models, and then the volumes of the actual hearts were determined by displacement after excision. The method, as performed by students, was found subject to an error of not more than 20 per cent. The volumes of the pericardial cavities of the normal animals were also found by making 'Plasticine' casts of the cavities after excision of the hearts. From these results it appears that the volumes of the heart and of the pericardial cavity of the abnormal animal are within the range to be expected, and that often the heart occupies only about half the volume of the pericardial cavity.

In the development of craniates, the pericardial cavity is first delineated from the perivisceral cœlum by the mesocardia. At this stage the pericardial cœlom is still in contact with the perivisceral cœlom by the dorsal and ventral parietal recesses of His. Afterwards the primary septum transversum is formed by proliferation of connective tissue on the anterior face of the developing liver[1]. It must be presumed that in this animal the liver rudiment initially developed asymmetrically in such a way that the aperture ventral to the right mesocardium was not occluded by connective tissue on the anterior face of the liver rudiment. Thus the abnormality represents a patent right ventral parietal recess of His. Goodrich[2] has shown that in *Scyliorhinus canicula* the dorsal apertures remain as a forked pericardio-peritoneal canal. The anterior end of this canal was clearly visible in the abnormal animal, although the forked posterior ends could not be traced.

A. S. Fuller

Department of Biology and Geology,
Norwood Technical College,
Knight's Hill, London, S.E.27.

[1] Nelsen, O. E., *Comparative Embryology of the Vertebrates*, 859 (Blakiston, New York, 1953).
[2] Goodrich, E. S., *J. Anat.* (Lond.), **53**, 1 (1918).

Shoaling Behaviour in a Mysid

Mysidium columbiae (Zimmer) is common in shallow water in Kingston Harbour, Jamaica. It is found during the day in shoals close to the mangroves, usually but not exclusively in the shade of the trees. The shoals can be seen swimming horizontally a few centimetres below the surface of the water in a manner similar to shoals of small fish, for which indeed they were at first mistaken. The behaviour of *Mysidium* proves to be a combination of fixed responses to external stimuli and social responses by which the shoals are built up and maintained.

A number of *Mysidium* placed in a large vessel of sea-water will form a shoal in a few minutes and swim around with their bodies oriented parallel with each other at a constant distance apart. Small shoals tend to join larger ones and *Mysidium* separated from the main shoal by a sheet of glass attempt to swim through the glass to join the shoal provided the latter is close enough for them to see it. The critical distance for shoaling by visual orientation is about 4 cm. at 150 ft.-candles illumination. *Mysidium* placed in a circular dish 18 cm. in diameter illuminated from above swim in one direction around it, keeping station with each other so that all those along a given radius tend to complete a circuit in the same time, those near the circumference swimming several times faster than those near the centre. The speed of swimming is photokinetically controlled, being proportional to the logarithm of the light intensity over a range of at least two logarithmic units of illumination. At very high intensities swimming tend to be inhibited and the mysids sink to the bottom, while in very dim light the shoal breaks up. Removal of both eyes abolishes both the photokinetic response and the ability to swim in a shoal. Some astonishingly high swimming speeds have been recorded in these

experiments up to 15·4 cm./sec., which is equivalent to about twenty times the animal's length in a second.

Light-adapted *Mysidium* are positively phototactic and swim towards a source of light. Dark-adapted ones, however, always swim to the bottom of the tank when illuminated irrespective of the position of the light. After removal of the pair of statocysts situated in the uropods they always swim away from a light source, to the surface when illuminated from below and to the bottom when illuminated from above.

The behaviour of *Mysidium columbiae* is remarkable in several respects. Unlike most mysids it is a 'bright-light' animal living close to the water surface under the noonday tropical sun, while in its shoaling behaviour it shows a precision and co-ordination of locomotion comparable with that seen in fish. In some respects, however, the social responses of *Mysidium* are not so well developed as in shoaling species of fish. Two or more fish will form a shoal instantly but small numbers of mysids will not. It seems that critical density or number of animals is required before they will form shoals, which then build up rather slowly, taking from 30 sec. to 5 min. to become well developed. Also a proportion estimated at about 5 per cent do not join the shoals, but swim freely in other directions or remain motionless on the bottom. *Mysidium* is more suitable than fish in some ways for an experimental study of shoaling, since it shows no apparent capacity to modify its behaviour as a result of experience.

D. M. Steven

Department of Zoology,
University College of the West Indies.

Evidence for a Sexual Phase in the Life-Cycle of an Amœba

The amœba have generally been thought to be devoid of a sexual phase in their life-history[1], though one earlier report[2], and a recent one[3], indicate a cycle involving fusion of flagellated gametes. This communication concerns evidence for sexuality in a diphasic amœba.

A flagellate ascribable to the genus *Heteramœba* and very near *H. globosa* Skuja[4] is a widespread, though by no means common, inhabitant of brackish supra-littoral rock pools. This organism, referred to here as *Heterochromonas clara* nom. prov., differs from *H. globosa* in being larger (30μ diam.) and in not having contractile vacuoles, but, like *H. globosa*, it has no stigma. It was abundant in August 1960 in the polluted pools at the base of the cliff below the large gannetry on Ailsa Craig, where it was feeding voraciously on green flagellates. Two dozen bacteria-free clones were isolated from this source using a strain of *Brachiomonas submarina* for food. One clone was set aside for nutritional work and the remainder were maintained in composite culture for reference. The amœbæ first appeared in the latter culture. They were unexpected since *Heterochomonas* is not recorded as being the stage in the life-cycle of an amœba; moreover, an isolate of *H. clara* from a similar habitat in Finland had been kept for ten years without sign of amœbæ.

Several of the amœbæ were isolated and washed singly and used to start cultures. Without exception every one of these cultures quickly gave rise to flagellates. Twenty flagellated cells were similarly isolated from one of the amœba isolates (145), the ensuing clones being designated 145/*a*–*t*. Amœbæ appeared in several of these within a few days, but others have given rise to no amœbæ after four months of maintenance. The latter, six in number, were then crossed with each other, that is, mixed in subculture two at a time in all combinations, for it seemed that the flagellate/amœba transformation might, in contrast to other diphasic amœbæ, be under genetic rather than environmental control. So indeed it proved, for with certain combinations amœbæ appeared within 24 hr. of the operation, while with others, and in the six parent clones, the populations remained uniformly flagellate (Table 1). Furthermore, the pattern of amœba-formation is consistent with the existence of two mating types among the clones, all of which, it will be recalled, are descended from the same amœba cell. This result is taken as indirect, though conclusive proof of a sexual phase in the life-cycle of this organism.

Table 1. Pattern of Amœba-formation in Bi-clonal Cultures

Clone	145*d*	145*k*	145*r*	145*f*	145*i*	145*n*
145*d*	—	—	—	*A*	*A*	*A*
145*k*	—	—	—	*A*	*A*	*A*
145*r*	—	—	—	*A*	*A*	*A*
145*f*	*A*	*A*	*A*	—	—	—
145*i*	*A*	*A*	*A*	—	—	—
145*n*	*A*	*A*	*A*	—	—	—

A, Formation of amœbæ within 24 hr.

The amœbæ are at first minute (7 × 30μ) and typically *limax*-like but later reach a size of 40 × 70μ; they have been observed to multiply by binary fission. Cysts (up to 25 × 30μ, smooth-walled) are found only in amœba cultures; they germinate to produce single amœbæ. Possible isogametic fusions between minute flagellates can be observed in mixed cultures, but this is open to an alternative interpretation since *H. clara* is phagotrophic and able to devour cells of its own size or larger and is not above resorting to cannibalism.

The following hypothetical conclusions may be drawn from these preliminary observations: (1) the amœbæ are diploid; (2) flagellated cells may be diploid or haploid; (3) only diploid cells can encyst; (4) haploid cells are of two mating types; (5) multiplication can occur both in the diploid and haploid states and can continue indefinitely in the latter.

Multiplication of flagellated cells of diphasic amœbæ is not unknown; it has been reported, for example, for *Tetramitus rostratus*[5], together with the suggestion of a sexual phase. It is not yet established that amœbæ of *H. clara* can multiply for an indefinite period without reversion to flagellates; nevertheless, considerable fluidity appears to exist in the balance between haploid and diploid phases of the life-cycle, the cycle being primitive on that count as compared with other sexual Sarcodina.

A cytological investigation of *H. clara* is being undertaken as an aid to a proper diagnosis of the organism and to a more detailed description of the life-history.

M. R. Droop

Marine Station,
Millport,
Scotland.

[1] Jepps, M. W., *The Protozoa, Sarcodina* (Oliver and Boyd, London, 1956).
[2] Jones, P. N., *Arch. Protistenk.*, **43**, 322 (1928).
[3] Cigarda, L. N., *Inst. Lombardo (Rend. Sci.)*, **92**, 431 (1958).
[4] Skuja, H., *Symb. bot. Upsaliens*, **9** (3), 1 (1948).
[5] Bunting, J., *J. Morph.*, **42**, 23 (1926).

Influence of Larval Environment on Adult Size and Fecundity in the Moth *Panaxia dominula* L.

The scarlet tiger moth, *Panaxia dominula*, is to-day found only in southern parts of the British Isles. Since it was at one time a little more widespread[1] the present distribution may be due to the action of environmental limiting factors rather than to the lack of opportunity to colonize more northern parts of the country. Evidence is presented here which supports this assumption.

The wing-span of moths caught in several English colonies was measured from the tip of the right wing to the centre of the thorax on pinned specimens. There is considerable variation in size between different colonies, insects from Devon being significantly larger than those from Berkshire (Table 1*a*). (For the difference between Axmouth and Cothill: males, $t = 10{\cdot}37$, $P < 0{\cdot}001$; females, $t = 10{\cdot}89$, $P < 0{\cdot}001$). When larvæ were collected late in development and reared to maturity under similar conditions it was not manifested (Table 1*b*). For the males, $t = 0{\cdot}23$, $P > 0{\cdot}80$; for females, $t = 2{\cdot}11$, $P = 0{\cdot}05$–$0{\cdot}025$. It seems likely, therefore, that the differences in size between wild-caught insects are determined primarily by environmental dissimilarities between the colonies rather than by the genetic constitution of the individuals in them.

The number of eggs produced by bred females was counted and their wing-span measured. When productivity is plotted against wing-span it is seen that there is a significant positive correlation between them in a sample of 22 from Cothill ($r = 0{\cdot}604$, $P < 0{\cdot}01$), and in a sample of 6 from Kingsdown, Kent ($r = 0{\cdot}852$, $P < 0{\cdot}05$). The correlation is just below the 5 per cent level for 28 specimens from Axmouth ($r = 0{\cdot}347$. With 28 points the 5 per cent value is 0·374). The data are shown in the graph (Fig. 1). It may be that in Nature environmental conditions do not affect the mean number of eggs to the same extent, but the range in size in the experimental régime was considerably less than that between naturally emerging insects from the Devon and Berkshire colonies, so that the results strongly suggest that size differences in them are accompanied by a corresponding variation in egg production. Similar data from other insect species are discussed by Klomp[2]. In this example the smallest individuals come from

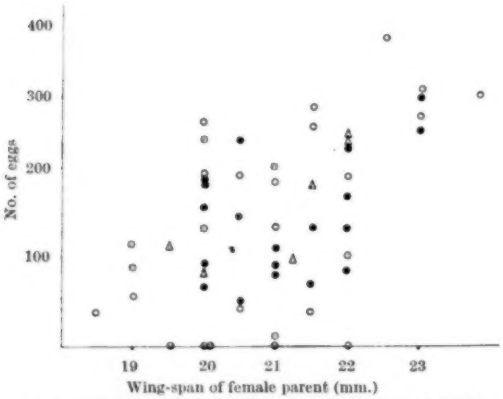

Fig. 1. Regression of number of eggs on wing-span of female parent. ○, Cothill; ●, Axmouth; Δ, Kingsdown. In this series the size was measured from wing-tip to the join of nervures 1 and 2

Table 1. *a*, Size in mm. (to nearest half mm.) from Tip of Wing to Centre of Thorax in Insects caught wild

	No.	Mean and standard deviation	Range
Cothill (Berks) ♂♂	27	23·30 ± 1·15	21·0–25·0
Cothill ♀♀	25	22·78 ± 0·98	21·0–24·5
Sheepstead Hurst (Berks)	15	24·10 ± 1·15	22·5–25·5
Bradfield (Berks)	4	25·75 ± 0·65	25·0–26·5
Newbury (Berks)	4	25·88 ± 1·32	24·0–27·0
Burgate (Hants)	8	25·81 ± 1·12	23·5–26·5
Axmouth (Devon) ♂♂	68	25·96 ± 1·07	23·5–28·5
Axmouth ♀♀	42	25·54 ± 1·04	23·0–27·5

Table 1. *b*, Measurements (to the nearest half mm.) on Adults Bred from Fourth Instar Larvæ

	No.	Mean and standard deviation	Range
Cothill ♂♂	16	24·81 ± 1·48	21·5–26·5
Cothill ♀♀	22	23·82 ± 1·66	22·0–27·0
Axmouth ♂♂	20	24·70 ± 1·42	22·5–27·5
Axmouth ♀♀	31	24·71 ± 1·28	21·5–27·5

colonies near the edge of the present range. They probably lay fewer eggs than those from south-west England, so that reduced productivity may be one of the limiting factors.

This work was carried out at the University of Oxford during the tenure of a grant from the Nature Conservancy.

L. M. Cook

Department of Zoology,
University of Leicester.

[1] Cook, L. M., *Entomologist*, **92**, 232 (1959).
[2] Klomp, H., *Arch. Neerland. de Zool.*, **13**, Supp., 319 (1958).

Localization of Esterase in the Cuticle of the Nematode *Ascaris lumbricoides*

The cuticle of nematodes, because of its obvious skeletal functions and its importance as a barrier to nematocides, has recently been the subject of much research. Bird and Deutsch[1] and Bird[2] have greatly increased our knowledge of the structure of the cuticle of *Ascaris lumbricoides* and various other workers have added to our knowledge of the chemical composition of the cuticle[3–5]. Although Fairbairn[6] states "it would seem wise to regard the integument as a fully integrated living tissue, rather than as an inert horny sheath" there has been no evidence to show that the cuticle of nematodes is metabolically active.

Frozen sections (50μ) of *Ascaris lumbricoides* (from pigs), fixed in 10 per cent neutral formol saline at 4° C. for 24 hr., were tested for non-specific esterase activity by two methods as described by Pearse[7] (1), the naphthol *AS* acetate – fast red *TR* salt and (2), the 5-bromo-indoxyl acetate methods.

A positive result was obtained, with both methods, in the homogeneous, or matrix, layer of the cuticle but not in any of the other layers. The colour was most intense at the anterior and posterior ends of the nematode. In transverse sections there is a narrow gap in the deposit of stain in the homogeneous layer at the lateral lines.

There was complete inhibition of colour formation after incubation with 10^{-2} *M* silver nitrate; 90–100 per cent inhibition after 10^{-4} *M* *E*600 (diethyl *p*-nitrophenyl phosphate); 50 per cent inhibition after 10^{-3} *M* cupric sulphate and 90–100 per cent inhibition after boiling for 15 min., but 10^{-4} *M* eserine appeared to have no inhibitory effect.

Lipids are known to be present in the cuticle of *Ascaris* and certain other nematodes[6,8,9]. Contrary to the findings of Bird[10], a weakly positive result for lipid was obtained with oil red *O* in the homogeneous layer of the cuticle. Thus the distribution of non-specific esterase coincides with the distribution of lipid in the homogeneous layer of the cuticle of

Ascaris. These findings provide strong evidence in favour of the view expressed by Fairbairn[6] and by Bird[10] that the cuticle of *Ascaris lumbricoides* is a metabolically active structure. The presence of at least one enzyme in the cuticle would indicate that more are present and it is possible that these enzymes are concerned with the laying down of more cuticle as the nematode increases in size after the final moult.

Esterase, inhibited by 10^{-4} *M* E600, has also been localized in the œsophageal glands, in the intestine, in the rectal glands, in the cœlomocytes, in the nervous system including the innervation processes of the body wall muscles, in the gonads, and as 'caps' of enzyme on fat globules in the hypodermis, in the dorsal, ventral and lateral lines, and in the muscles. The cells of the intestine have been found to be merocrine in that they discharge esterase through the striated border without undergoing any marked changes. The presence of esterase in the innervation processes of the body wall muscles, but not in the non-contractile part of the muscle other than on fat globules, would suggest that this esterase is a cholinesterase or an esterase serving a similar function in nerve transmission.

D. L. Lee

Molteno Institute,
Downing Street, Cambridge.

1 Bird, A. F., and Deutsch, K., *Parasitology*, **47**, 319 (1957).
2 Bird, A. F., *Parasitology*, **48**, 32 (1958).
3 Hobson, A. D., *Parasitology*, **38**, 183 (1948).
4 Fairbairn, D., *Exp. Parasit.*, **6**, 491 (1957).
5 Fairbairn, D., *Nematology: Fundamentals and Recent Advances with Emphasis on Plant Parasitic and Soil Forms*, Chapter 30, edit. by Sasser, J. N., and Jenkins, W. R. (Univ. of North Carolina Press, 1960).
6 Fairbairn, D., *Canad. J. Biochem. Phys.*, **34**, 39 (1956).
7 Pearse, A. G. E., *Histochemistry: Theoretical and Applied*, second ed. (Churchill, London, 1960).
8 Cavier, R., Savel, J., and Monteoliva, M., *Bull. Soc. Chim. Biol.*, **40**, 177 (1958).
9 Simmonds, R. A., *Exp. Parasit.*, **7**, 14 (1958).
10 Bird, A. F., *Exp. Parasit.*, **6**, 383 (1957).

ENTOMOLOGY

Effect of Chlorinated Hydrocarbon Insecticides on Insect Choline Acetylase, Condensing Enzyme and Acetylkinase

The role of acetylcholine in the transmission of nerve impulses is by no means as well established in insects[1,2] as it is in mammals, but the presence in insect nervous tissue of large quantities of acetylcholine[3] and of enzymes capable of its rapid synthesis[4] and hydrolysis[5] is clearly established.

It has also been shown that chlorinated hydrocarbon insecticides have an effect on nerve conduction in insects[6,7] and on their acetylcholine-levels[8].

That these insecticides kill insects as a direct or indirect consequence of their effect on nerve conduction has not been clearly demonstrated but it still remains the most likely explanation of their mode of action.

The work reported here was carried out in an attempt to determine what effect these insecticides have on the synthesis of acetylcholine by insects, being part of a re-investigation of the effects of chlorinated hydrocarbon insecticides on the synthesis, hydrolysis and action of acetylcholine in insects.

Dieldrin-susceptible house-flies *Musca domestica* L. were reared at 25° C. on a larval diet of 120 gm. powdered whole milk, 120 gm. powdered dried yeast and 24 gm. agar powder made up to 1,200 ml. with water. The adults were fed on glucose and on a 5 per cent aqueous glucose solution. Tissue homogenates were prepared using a small Waring blender. 300 adult *M. domestica*, 5–10 days old, were homogenized at 0° C. in 10 ml. 0·25 *M* sucrose for 5 min. Alternatively, the flies were decapitated, after anæsthesia by cooling, and the heads only were homogenized (300 heads/10 ml. 0·25 *M* sucrose solution) as above. In both cases the resultant homogenates were filtered at the pump through muslin to remove the larger pieces of cuticle, and made up to 10 ml.

To determine the normal rate of synthesis of acetylcholine using either sodium acetate or sodium citrate as acetyl donor, reaction mixtures were prepared as follows and incubated in a water-bath at 30° C : 2·0 ml. homogenate; 0·2 ml. coenzyme *A* (1 mgm./ml. Light and Co., Ltd.); 0·2 ml. $6{\cdot}6 \times 10^{-2}$ *M* adenosine triphosphate; 0·2 ml. 0·1 *M* L-cysteine; 0·4 ml. 16×10^{-3} *M* eserine sulphate, or preferably, 0·4 ml. 0·01 *M* tetraethylpyrophosphate (TEPP) (both cholinesterase inhibitors); 0·4 ml. 0·4 *M* choline chloride; 0·4 ml. 0·1 *M* phosphate buffer *p*H 7·0; 0·2 ml. sodium fluoride (10 mgm./ml. phosphatase inhibitor).

To test the effect of insecticide, sufficient was dissolved in 0·2 ml. acetone to give a final concentration in the reaction mixture of 10^{-3} *M*. Control experiments were carried out using 0·2 ml. acetone added to the reaction mixture. 0·4-ml. aliquots were removed at intervals and their acetylcholine content measured using Hestrin's method[9].

The activity of the fly preparations, with and without acetone[10] and irrespective of whether acetate or citrate was the actual donor, was 0·7 μgm. acetylcholine synthesized/head/hr. and 1·3 μgm. acetylcholine whole fly/hr. The activity when expressed per gram of fresh tissue was 567 μgm. acetylcholine/gm./hr. for fly heads and 120 μgm. acetylcholine/gm./hr. for whole flies. On the addition of the chlorinated hydrocarbon insecticides *pp'*DDT (dichlor diphenyl trichlorethane), dieldrin, heptachlor and lindane in 0·2 ml. acetone and at a final concentration of 10^{-3} *M* the activity of the preparations remained unaltered.

Smallman[4], in his demonstration of acetylcholine synthesis in the blowfly *L. sericata*, found that the rate of acetylcholine synthesis from acetate by acetylkinase was considerably greater than that from citrate by the condensing enzyme working in reverse. He also demonstrated that choline acetylase is much more active than either condensing enzyme or acetylkinase and that these latter enzymes appeared to control the rate of synthesis of acetylcholine.

Our results on *M. domestica*, in which preparations of similar activity were used, show that as in *L. sericata* both pathways for the synthesis of acetylcoenzyme *A* are present, but that in *M. domestica* the rate of synthesis from acetate or citrate was the same. This indicates that either choline acetylase is less active than the other two enzymes and thus acts in a rate-controlling capacity, or that in the house-fly as in the guinea pig both pathways are equally available.

Lewis *et al.*[8] have shown that in cockroaches poisoned and prostrated by *pp'*DDT and in cockroaches physically stimulated to prostration there is a rise in their acetylcholine-levels. He postulates that this is due to the release of acetylcholine from a bound form at abnormal sites in the nervous system since his results also showed that cholinesterase was not inhibited by the above treatments. Our finding that the rate of synthesis of acetylcholine in *M. domestica* is not affected by the chlorinated hydrocarbon insecticides *pp'*DDT, dieldrin, heptachlor and lindane supports Lewis's suggestion since it excludes

the possibility that the increased acetylcholine-levels could be due to an increased rate of production of acetylcholine due to chemical or physical stimulation.

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Jennifer Rothschild
G. F. Howden

Imperial College Field Station,
Ashurst Lodge, Silwood Park,
Sunninghill, Ascot, Berks.

[1] Colhoun, E. H., *Nature*, **182**, 1378 (1958).
[2] Hopf, H. S., and Taylor, R. T., *Nature*, **182**, 1381 (1958).
[3] Lewis, S. E., *Nature*, **172**, 1004 (1953).
[4] Smallman, B. N., *J. Physiol.*, **132**, 343 (1956).
[5] Richards, A. G., and Cutkomp, L. K., *J. Cell and Comp. Physiol.*, **26**, 57 (1945).
[6] Kearns, C. W., *Ann. Rev. Ent.*, **1**, 123 (1956).
[7] Dahm, P. A., *Ann. Rev. Ent.*, **2**, 247 (1957).
[8] Lewis, S. E., Waller, J. B., and Fowler, K. S., *J. Inst. Physiol.*, **4**, 128 (1960).
[9] Colowick, S. P., and Kaplan, N. O., *Methods in Enzymology*, **1**, 649 (Academic Press, Inc., New York, 1955).
[10] Colhoun, E. H., *Nature*, **189**, 309 (1961).

Substances controlling the Rate of Beating of the Heart of *Periplaneta*

A paper now in the press[1] shows that the heart accelerating factor from the corpus cardiacum of the cockroach *Periplaneta americana* described by Cameron[2,3] acts by stimulating the pericardial cells to produce a second substance. It is this pericardial cell factor which is responsible for the direct stimulation of the heart. This communication describes the properties of the two substances.

The factor from the corpus cardiacum appears to be a peptide or protein. Heating extracts (prepared by grinding corpora cardiaca in insect Ringer or in distilled water) on a boiling water-bath for 5 min. results in a slight increase in activity as measured on the isolated cockroach heart. More prolonged heating has no further effect on the activity. The activity of extracts is unaffected by evaporation to dryness at room temperature and resuspension in distilled water. The dried residue can be extracted with *n*-butanol, acetone or diethyl ether without loss of activity. The active factor appears to be soluble in 80 per cent ethanol, but treatment of the dried residue with absolute ethanol destroys the activity. Incubation of extracts with trypsin at *p*H 8 or pepsin at *p*H 3 results in complete inactivation within 60 min. at 37° C.

However, Cameron[2,3] has suggested that the active substance from the corpus cardiacum is an *o*-diphenol. Since Cameron's extraction technique involves the use of absolute ethanol as a solvent for the active principle, his material must be different from the material under study here. Further confirmation of the non-identity of the two materials is obtained from a consideration of the behaviour on paper chromatograms of distilled water extracts of corpora cardiaca and extracts prepared according to the method of Cameron. Using *n*-butanol/acetic acid/water (4 : 1 : 5) as a solvent, the activity from corpora cardiaca extracted in distilled water is associated with a ninhydrin-positive spot at R_F 0·23. Similar chromatograms using Cameron's technique have yielded highly variable results; it is sufficient to say here that no activity was ever recovered from the area around R_F 0·23. Further, a number of spots, some sensitive to ninhydrin, others to ferric ferricyanide, appeared at higher R_F values. None of these spots could be detected on chromatograms developed from distilled water extracts. Using phenol as a solvent, it has not been possible to detect a spot on chromatograms developed from distilled water extracts which turn pink after treatment with 0·44 *M* potassium ferricyanide. On the other hand, such spots appear on chromatograms in which Cameron's technique was used to extract the corpora cardiaca. It is worth noting that Cameron preferred to extract the *o*-diphenol from whole larvae of *Tenebrio*, as the extraction from the corpora cardiaca of *Periplaneta* was difficult and unreliable[2].

The material from the pericardial cells appears to be an indolalkylamine. The isolated cockroach heart is very sensitive to serotonin: the threshold for stimulation is below 10^{-7} *M*. Extracts of pericardial cells activated by the factor from the corpus cardiacum have a slight and transient melanophorotropic effect on the melanocytes of the skin of the frog *Rana temporaria*, a property shared by the indolalkylamines[4]. The action of extracts of the corpus cardiacum on the isolated heart of *Periplaneta* can be potentiated by first exposing the heart to a concentration of 100 μgm./ml. of iproniazid, an inhibitor of the enzyme monoamine oxidase. Further, 2-bromolysergic acid diethylamide, an antagonist of indolalkylamines, markedly reduced the effect of extracts of corpus cardiacum when applied at a concentration of 10^{-5} *M*. These results are taken as evidence that an indolalkylamine is involved in the action of the corpus cardiacum on the heart.

If activated pericardial cells are extracted by a technique designed to separate amines[5], a pharmacologically potent extract results which possesses a single ultra-violet absorption peak at 266 mμ. Similar extracts of inactive pericardial cells, on the other hand, are pharmacologically inert, and possess an absorption peak at 275 mμ, with a shoulder at 286 mμ. This probably represents an inactive precursor of the active factor.

Extracts of active pericardial cells have been run on paper chromatograms using *n*-butanol/acetic acid/water (4 : 1 : 5) as a solvent. The pharmacological activity on such chromatograms is located in the vicinity of R_F 0·5. After treatment with ninhydrin-acetic[6], a spot exhibiting blue-green fluorescence in ultra-violet light appears at R_F 0·5. This spot is absent from chromatograms of extracts of inactive pericardial cells. The test is diagnostic for derivatives of tryptamine. It is worth noting that the active factor from the opaque accessory glands of male insects[7] can be extracted by the same technique and shows a strong absorption peak at 266 mμ.

Finally, active and inactive pericardial cells have been examined histologically. Active cells are considerably larger and contain more secretion droplets. A greater proportion of the active cells exhibit argentaffin granules, a characteristic feature of cells which produce indolalkylamines.

I am indebted to Prof. V. B. Wigglesworth for his continued interest and for the provision of facilities.

K. G. Davey

Zoology Laboratory,
Downing Street, Cambridge.

[1] Davey, K. G., *Gen. Comp. Endocrinol.* (in the press).
[2] Cameron, M. L., Dissertation, Cambridge (1953).
[3] Cameron, M. L., *Nature*, **172**, 349 (1953).
[4] Davey, K. G., *Canad. J. Zool.*, **38**, 717 (1960).
[5] Weissbach, H. H. Waalkes, T. P., and Udenfriend, S., *J. Biol. Chem.*, **230**, 865 (1958).
[6] Jepson, J. B., and Stevens, B. J., *Nature*, **172**, 772 (1953).
[7] Davey, K. G., *Canad. J. Zool.*, **38**, 39 (1960).

CYTOLOGY

Chromosomes of the Orang-Utan (*Pongo pygmaeus*)

APART from the data on the chromosome number of chimpanzees[1,2] there are no other data available concerning the number and the morphology of the chromosomes of other anthropoids. While engaged on a programme of research on the phylogenetic relationships of primates, I had the opportunity of studying the chromosomes of two orang-utans (male and female, *R.* 33 and *R.* 34) in the Zoological Gardens of Rome.

Since it is planned to publish at a later date morphometric comparative data for most species of primates, in this communication only a description of the chromosomes is given.

The usual techniques for the study of chromosomes in tissue cultures were followed[3]. The chromosome counts are summarized in Table 1.

Table 1

$2n$ =	46	47	48	49
*R.*33	1	2	16	1
*R.*34	—	3	21	—

The diploid number of the chromosomes of *Pongo pygmaeus* can thus be considered $2n = 48$; the same as that of the chimpanzee.

Fig. 1 shows the reconstruction, in pairs, of male and female chromosomes. They are arranged in a preliminary order; a more satisfactory grouping will become possible when more data become available.

In Table 2 the autosomes are classified according to the position of the centromeres:

Table 2

Metacentric	Submetacentric	Telocentric
1, 17, 20, 21, 22	3, 4, 5, 7, 11, 12, 13, 16, 23	2, 6, 8, 9, 10, 14, 15, 18, 19

The *X*-chromosome is metacentric; the *Y*-chromosome has a large achromatic zone.

The orang-utan's karyotype shows certain similarities to the human one. Chromosomes 1, 4, 5, 6 and 7 closely resemble chromosomes 1, 4, 5, 6 and 7 of man, according to the nomenclature of the Denver human chromosomes study group. The three pairs of telocentric chromosomes of man (13, 14, 15) correspond morphologically and dimensionally with 10, 14 and 15 of the orang-utan. The pair 23, moreover, looks like chromosome 22 of man, having one of the two arms heterochromatic. As regards the sex chromosomes, the *X*-chromosome of the orang-utan shows a strong resemblance to the human one, while the *Y*-chromosome differs by having a large achromatic portion.

A more careful study by means of metric analysis[3] will be required, however, before establishing such similarities.

My thanks are due to the director of the Rome Zoological Gardens, Prof. E. Bronzini, for enabling me to study these animals.

BRUNETTO CHIARELLI
Istituto di Genetica,
Università di Pavia.

[See also p. 225 of this issue.]

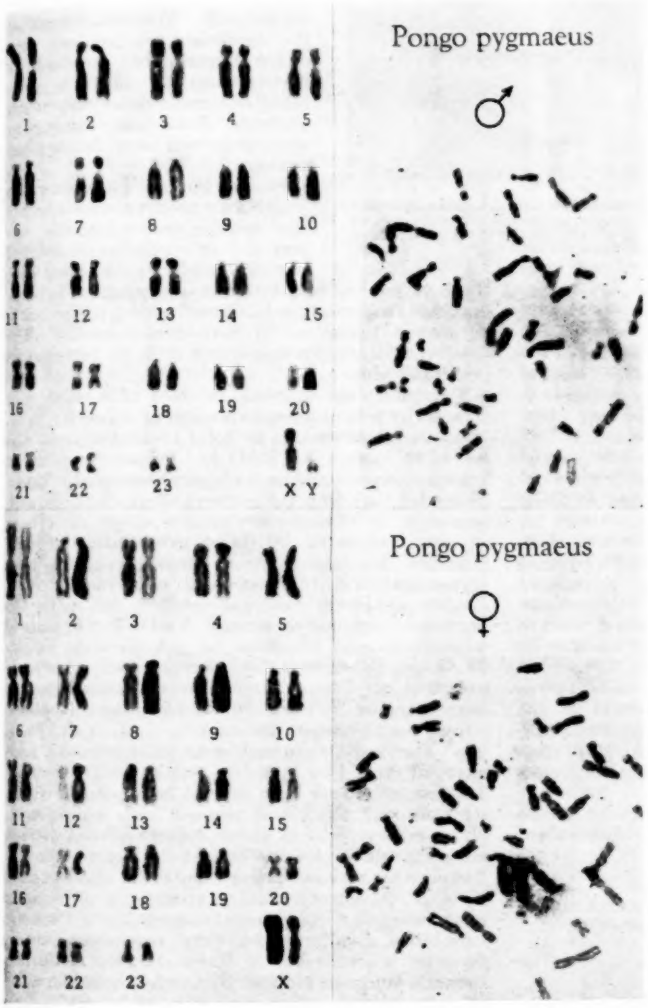
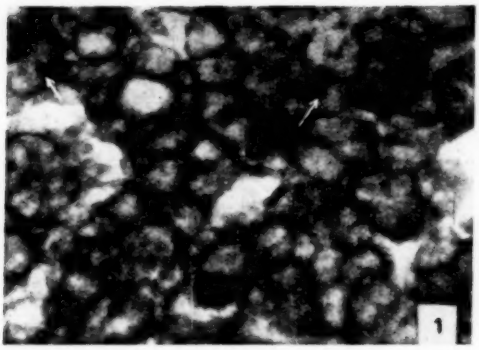


Fig. 1

[1] Yeager, C. H., Painter, T. S., and Yerkes, R. M., *Science*, **91**, 74 (1940).
[2] Young, W. J., Merz, T., Ferguson-Smith, M., and Johnston, A. W., *Science*, **131**, 1672 (1960).
[3] Chiarelli, B., Nuzzo, F., and De Carli, L., *Atti A.G.I.*, **5**, 263 (1960).

Nucleolar Succinic Dehydrogenase in Mouse Mammary Carcinoma Cells

THE oxidative enzyme succinic dehydrogenase is principally localized[1] in the mitochondria and absent in the nuclei of all mammalian cells. However, the enzyme had been localized in the nuclei of bird



Figs. 1 and 2. Arrows indicate the nucleoli of malignant cells in histological section and whole-cell suspension from mouse mammary carcinoma, respectively (pyronin methyl green). Fig. 3. Arrow indicates the nucleolar formazan deposition in the same type of malignant cells in suspension. (All × *c.* 400)

erythrocytes which according to Brachet[2] may be due to absence of mitochondria in these cells.

Nucleolar succinic dehydrogenase has been shown to be present, in normal rat liver cells[3] and also in the malignant cells[4] from epidermoid carcinoma of human cervix. Now nucleolar localization of this enzyme has been confirmed in malignant cells of a different biological material (mouse mammary carcinoma).

A suspension of intact whole cells in 0·1 *M* ice-cold phosphate buffer of *p*H 7·6 was prepared from spontaneous solid mammary carcinoma arising in *DBA* mice, which removed the endogenous substrates for succinic dehydrogenase. A portion of this suspension was smeared on glass slides for staining with pyronin methyl green and cellular sediment from the remaining portion was stained for succinic dehydrogenase[5]. A small piece of tissue from the tumour was fixed in carnoy, sectioned at 5μ thickness and stained by pyronin methyl green.

Photomicrographs show a close similarity as regards nucleolar shape, size and position of the pyronin methyl green preparations and formazan-stained nucleolar bodies (Figs. 1, 2 and 3). More than a thousand malignant cells were investigated on each slide.

Thanks are due to Dr. S. K. Brahma for active co-operation and to N. Saha for the photomicrography.

P. De
R. Chatterjee
S. Mitra

Cell Research Department,
Chittaranjan National Cancer
Research Centre,
Calcutta, 26.

[1] Pearse, A. G. E., and Scarpelli, D. G., *Nature*, **181**, 702 (1958).
[2] Brachet, J., *Biochemical Cytology*, 94 (Academic Press, Inc., New York, 1957).
[3] De, P., Chatterjee, R., and Mitra, S., *Nature*, **191**, 925 (1961).
[4] De, P., Chatterjee, R., and Mitra, S., *J. Histochem. and Cytochem.* (in the press).
[5] De, P., Chatterjee, R., and Mitra, S., *Exp. Cell Res.*, **24**, 158 (1961).

Chromosomal Lesion associated with Carcinogen-induced Tumours in Mice

Primary tumours induced by polycyclic hydrocarbons have been examined by several investigators to evaluate the importance of karyotype modification in carcinogenesis[1–3]. A significant number of such tumours has been characterized by cells with aneuploid chromosome numbers. In a study of 14 fibrosarcomas induced by methylcholanthrene in a variety of F_1 hybrids of inbred mouse strains, Hellström[2] reported that only 4 tumours contained predominantly diploid cells. The cells comprising the remaining ten tumours were either hyperdiploid, triploid, or hypertetraploid. Marked variability in chromosomal pattern was observed from one tumour to another as well as within any one tumour.

Studies in our laboratory are under way to determine whether the cytogenetic variability observed in carcinogen-induced tumours might be correlated with the inducing hydrocarbon, sex and strain differences in tumour incidence, or the latent period, antigenicity, or growth behaviour of individual tumours. The results of these investigations will be reported in detail elsewhere.

Tumours were induced, in mice of a variety of strains, by subcutaneous injection of either 0·1 mgm. 20-methylcholanthrene or 3,4-9,10-dibenzpyrene dissolved in sesame oil (Table 1). Primary tumours or tumours maintained in isologous passage by trocar pieces for various periods were examined. Cells were prepared for chromosome studies either after brief *in vitro* culture or by direct examination of solid tumours. For tissue culture, tumour fragments were trypsinized and the recovered cells incubated in Eagle's minimum essential medium containing 10 per cent fœtal bovine serum. Variability in time of attachment and initiation of cell division, usually 24–48 hr., determined the interval in culture prior to examination. Squash preparations of metaphase cells were obtained by standard colchicine and hypotonic citrate pretreatment, aceto-orcein fixation and staining. For direct examination of solid tumours, mice were injected, 1 hr. prior to death, with colchicine. Tumour fragments were minced in hypotonic citrate solution and fixed and stained with aceto-orcein. Short-term growth in tissue culture offered distinct advantages over the use of material derived directly from the mouse since larger numbers of dividing cells, as well as chromosomal preparations of superior morphological detail, were obtained. The cytogenetic variability manifested by these carcinogen-induced tumours is illustrated in Table 1. No distinction between tumours induced by methylcholanthrene or dibenzpyrene is apparent. Similar to Hellström's findings, those tumours represented by a true diploid modal chromosome number were seen to be composed of some aneuploid cells.

The presence of chromatid breaks, occurring regularly in a certain proportion of the cells of 20-methylcholanthrene- and 3,4-9,10-dibenzpyrene-induced tumours, has been observed in preparations directly from solid tumours as well as from cultured material (Fig. 1). Table 2 indicates the frequency of cells with such breakage for several tumours of each carcinogen group. Only those cells selected as suitable for accurate chromosome counts were scored for breakage. Characteristic breaks were noted in cultured material which was not subjected to colchicine. Similar lesions have not been seen in other murine cells,

Table 1. Distribution of Chromosome Numbers of Metaphase Cells in Carcinogen-induced Tumours

Table 1. Distribution of Chromosome Numbers of Metaphase Cells in Carcinogen-induced Tumours

	Tumour no.	Host strain	Sex	Passage generation	Chromosome numbers 38	39	40	41	42	43	44	45	46	47	48	49–69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89–97	98	99	100	101	102	103	104	105–177	Total cells	Tumour characterization
20-Methylcholanthrene	*M*–I	*BALB/c*	♀	6			4*	19	12	6																		2	1	1	3		1										*162*	50	Hyperdiploid
	M–II	*BALB/c*	♀	2				1																				2	2	1	2		1	1	1								*163, 168*	15	Hypertetraploid
	M–IV	(*I* × *C3H*)F_1	♀	Primary			9	17	1	1	1													2																			31	Diploid
	M–V	*BALB/c*	♀	3				12	18	7																			1		1		1										40	Hyperdiploid
	M–XII	*BALB/c*	♀	1		2	1	3	18	9	2	1	1															3														40	Hyperdiploid
	M–XIV	*BALB/c*	♂	Primary			3	5	5	2						*52†, 60*	1												2	1			1				1						*136*	24	Diploid-hyperdiploid
3,4-9,10-Dibenzpyrene	*D*–I	(*C3H* × *BALB/c*)F_1	♂	Primary			19	6	1	1																		1	1	1													30	Diploid
	D–II	*BALB/c*	♀	Primary		1		3	5	5	3	1																															18	Hyperdiploid
	D–V	*BALB/c*	♀	Primary				2	5							1 57						1	1	2	1																		119, 154, 155	17	Hyperdiploid-hypotetraploid
	D–VI	*BALB/c*	♂	Primary				1								*60, 61, 69*	2		2	3	2	1	3	3	2	3	5	1						1		*92, 95*	2	3		3	2	2	1	*109, 125*	49	Near-tetraploid
	D–VII	(*I* × *C3H*)F_1	♂	Primary															1	1	2			1	3	4	1		4	1	3	3		1								*157*	26	Hypertetraploid
	D–X	(*C3H* × *BALB/c*)F_1	♀	Primary				2	1	1						57			1	4	1	1	1	1	1											1	*92, 99*							*177*	19	Hypotetraploid

* Figures refer to number of cells counted. † Numbers in italics represent actual chromosome number per cell.

Table 2. Frequency of Chromatid Breakage in Metaphase Cells of Carcinogen-induced Tumours

	Tumour No.	No. of cells examined	No. of cells with breaks	Frequency (per cent)
20-Methylcholanthrene	*M*–I	50	25	50
	M–II	15	9	60
	M–IV	31	0	—
	M–V	40	6	15
	M–XII	40	4	10
	M–XIV	24	10	42
3,4-9,10-Dibenzpyrene	*D*–I	30	6	20
	D–II	18	7	39
	D–V	17	4	24
	D–VI	49	7	14
	D–VII	26	14	54
	D–X	19	8	37

from normal or tumour tissues, cultured in our laboratory.

Although the chromosomal discontinuity appears to be 'complete' most frequently, a thin interconnecting thread is occasionally seen. Rarely the break in the chromatid arm is only partial. In more than half the total number of metaphase cells with this chromatid lesion, only one break per cell was found. Higher numbers of breaks, up to 5 per cell, occurred with decreasing frequency. The number of breaks in each cell is apparently not related to ploidy. Breaks were present with a relatively high frequency in tumour *M*–I, which was originally examined in its 6th transplant generation. This tumour was again studied in its 11th passage generation and the breakage frequency was found to be similar.

At this time, we feel that no preferential breakage sites characterize these tumours. We have noted that for any one tumour there is occasional evidence of breaks occurring at comparable sites on the chromosomes of different cells. Investigations are under way to clarify this point; elucidation, however, will be made difficult by the lack of individuality of the mouse chromosome, in addition to the well-known occurrence of cryptic structural changes.

Whether or not the portion of the chromatid arm distal to the break remains attached through the next cell-division cycle is not known. Such a process could occur by means of a non-visualized chromosome matrix, or by a process of re-healing. That the chromatid fragment is at least sometimes lost was apparent. A portion of a metaphase chromatid was occasionally missing or a chromatid fragment was sometimes found distant from a deleted chromosome. At times the fragment was seen 'paired' to the sister chromatid on the side opposite the proximal portion

Fig. 1. Metaphase cell from tumour *M*–I, containing 43 chromosomes. Two chromatid breaks are demonstrated in area outlined. (× 1,760)

of the broken chromatid. The possible contribution of the observed chromatid breakage to structural changes within the chromosomal complement will require further analysis.

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June Lee Biedler
Lloyd J. Old
Donald A. Clarke

Division of Experimental Chemotherapy,
Sloan-Kettering Institute for Cancer Research,
Sloan-Kettering Division,
Graduate School of Medical Sciences,
Cornell University Medical College,
Rye, New York.

[1] Bayreuther, K., *Nature*, **186**, 6 (1960).
[2] Hellström, K. E., *J. Nat. Cancer Inst.*, **23**, 1019 (1959).
[3] Stich, H. F., *J. Nat. Cancer Inst.*, **25**, 649 (1960).

GENETICS

Effect of Kinetin on the Fertility of some Strains of *Neurospora crassa*

In experiments with *Neurospora crassa*, some strains when crossed are highly infertile and, therefore, further genetic analysis is difficult. This communication reports the effects of an addition of kinetin (6-furfurylamino-purine)[1] on the fertility of some strains of *Neurospora crassa* which are normally highly infertile.

The strains used were all derived from the Abbot and/or Lindegren wild-types[2] and had become infertile as a result of either ultra-violet irradiation (designated *A* in Table 1) or of selection for ascospore size (Lee, B. T. O., unpublished results) (designated *B* in Table 1).

Crosses were set up between various strains of the *A* and *B* series on a medium favouring sexual reproduction with kinetin (0–10 p.p.m.) added. The fertility was assessed after incubation for 15 days at 25° C. Each treatment was replicated five times. The fertility was measured by counting the number of perithecia per slope and ascospore production assessed on an arbitrary scale 0–10, in which 0 is equivalent to a complete lack of ascospore production and 10 corresponds to normal wild-type production of ascospores. The results are given in Table 1. It is evident that the perithecial production in eight of the nine crosses used is greatly increased by the presence of kinetin. Estimates of ascospore production follow a similar trend. It is notable that kinetin had no effect on the cross *B* 21 × *B* 14.

Table 1. Perithecial and Ascospore Production with Added Kinetin

Cross		Kinetin (p.p.m.) 0·0	0·5	1·0	2·0	4·0	10·0
A 13 × *A* 18	P*	64	122	128	154	146	152
	A†	2	2	3	3	4	3
A 12 × *A* 13	P	60	104	116	102	122	106
	A	2	4	4	4	4	3
A 19 × *A* 14	P	12	96	154	252	206	212
	A	1	3	4	6	5	5
A 21 × *A* 16	P	26	80	72	104	92	88
	A	1	4	4	5	4	4
B 17 × *B* 19	P	4	82	104	84	158	196
	A	0	2	2	2	4	4
B 21 × *B* 14	P	8	16	24	14	22	18
	A	1	1	1	1	1	1
B 1 × *B* 3	P	20	34	106	66	74	86
	A	1	2	3	1	2	3
B 1 × *B* 7	P	5	76	94	106	118	126
	A	0	2	3	3	4	3
B 16 × *B* 8	P	8	76	108	92	106	122
	A	0	2	3	2	4	4

* P = No. of perithecia produced per slope (mean of 5 replicates).
† A = Estimated ascospore production per slope (mean of 5 replicates).

It is thought that this effect of kinetin on the crossing behaviour of *Neurospora crassa* might assist in the study of many strains the infertility of which on crossing has hitherto prevented a genetic analysis.

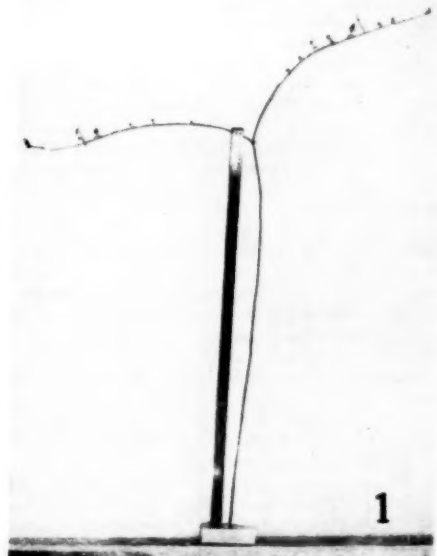
Barry O. Lee

Botany School,
University of Melbourne.

[1] Miller, C. O., *et al.*, *J. Amer. Chem. Soc.*, **78**, 1375 (1956).
[2] Pateman, J. A., *Heredity*, **13**, 1 (1959).
[3] Westergaard, M., and Mitchell, H. K., *Amer. J. Bot.*, **34**, 573 (1947).

Haploidy in Jute (*Corchorus olitorius* L.)

Haploidy was first reported to occur in *Datura stramonium* by Blakeslee *et al.*[1]. Since then, haploids occurring spontaneously or induced under artificial conditions have been recorded in many flowering plants[2]. As far as we are aware, haploidy in *Corchorus* has not yet been reported. Datta[3] reported a case of



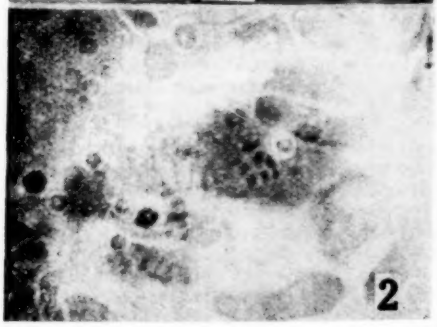


Fig. 1. The haploid *Corchorus olitorius* (*JRO*-632) plant. (× 1/14)
Fig. 2. Seven somatic chromosomes in the root tip cell. (× 1,700)

polyembryony in *Corchorus* without, however, any cytological analysis of the twin seedlings.

From a selection of X-ray-irradiated progenies of 70,000 r. treatment of the X_3 generation of *Corchorus olitorius* (*JRO*-632) in 1960, seven variants were found from a culture of healthy, vigorously growing normal plants. These plants with comparatively slender stem and small leaves were very late-flowering and the pod was small and stumpy. Among these variants, one plant was characterized by the presence of a very short and slender stem (Fig. 1) (length 97·5 cm.; thickness 5·2 mm.) and bearing a few flowers, from which only nine mature pods developed, measuring 1–1·5 cm. in length and 4–5 mm. in breadth. Six of the pods were empty. In the remaining three pods, one to three full seeds and two to three shrivelled seeds were obtained.

In most of the anthers, unseparated tetrads with collapsed walls were found. The anthers of the flowers did not dehisce; pollen sacs were empty with collapsed walls, though two full grains, taking stain in iodine, were found in a flower.

Of the seven full seeds, which are similar morphologically to the seeds of normal plants, three were germinated; from these, chromosome investigations were carried out. In the orcein squash of root-tip meristem of all the three seeds, seven chromosomes were counted from the metaphase plates (Fig. 2).

The seeds having haploid number of chromosomes may have arisen by parthenogenesis of a cell from the female gamete[4].

K. T. Jacob
Subir Sen

Jute Agricultural Research Institute,
Barrackpore,
West Bengal.

[1] Blakeslee, A. F., Belling, J., Fernham, M. E., and Bergner, A. D., *Science*, **55**, 646 (1922).
[2] Kehr, A. E., *J. Heredity*, **42**, 107 (1951).
[3] Datta, R. M., *Sci. and Cult.*, **25**, 642 (1960).
[4] Chandrasakharan, S. N., and Parthasarathy, S. R., *Cytogenetics and Plant Breeding* (P. Varadachary and Co., India, 1948).

VIROLOGY

Transmission of Lapinized Rinderpest Virus by Contact between Rabbits

Rabbits are readily infected with lapinized rinderpest virus administered parenterally, but contact infections are alleged not to occur[1,2]. Protocols, however, have not been published yet.

Eight groups of twenty rabbits were randomly paired and each pair was closely confined in a cage measuring 45 × 45 × 45 cm. One rabbit of each pair was given 1,000 ID_{50} of lapinized rinderpest virus intravenously. Rectal temperatures were recorded daily. In the first trial insufficient attention was paid to the risk of transmission by clinical thermometers for we have found since that lapinized rinderpest virus can be recovered from the faeces of infected rabbits and that intrarectal instillation of the virus readily infects. In subsequent trials different thermometers were used to record the temperatures of inoculated and uninoculated rabbits.

Close contact was maintained until the inoculated rabbit died. Thereafter the uninoculated rabbits were observed for a further period of 10 days, after which they were killed and examined for signs of rinderpest. In the final trial the period of observation was extended to 30 days. Uninoculated rabbits which developed a fever were killed and examined. Tissues from those having suggestive lesions were collected, emulsified and inoculated into other rabbits.

All eighty inoculated rabbits were infected and all died. In the first trial one of the in-contact rabbits contracted rinderpest and we now believe that transmission occurred rectally via the thermometer. In the subsequent trials two in-contact rabbits had suggestive lesions but virus was not recovered from their tissues and infection was not considered to have occurred.

Our findings have confirmed the earlier statements that contact transmission of lapinized rinderpest virus does not occur between sick and healthy rabbits. Direct contact, nevertheless, is the primary mode of transmission of wild strains of rinderpest and all authenticated outbreaks in previously disease-free countries have been linked to the importation of live animals from endemic areas[5]. Lapinized rinderpest virus has undergone many serial passages in rabbits and in the process has become attenuated for cattle and, at the same time, highly virulent for rabbits. It has also lost its ability to transmit by contact. In this respect it simulates the Kabete *O* strain of rinderpest virus which has been 'needle-passaged' in cattle for many years and which does not spread readily between sick and healthy cattle although it is highly virulent for cattle[3]. Presumably the route of infection with both strains has favoured the selection of mutants deficient in natural invasiveness. Both strains still, however, induce gross changes in the alimentary tract[4].

Recognition of the possibility of selecting mutants of virulent strains of virus which do not spread by contact should stimulate the search for a selection of similar mutants of attenuated strains intended as live virus vaccines.

We wish to thank Mr. H. R. Binns, director of the East African Veterinary Research Organization, for his advice and encouragement and permission to publish this communication.

G. R. Scott
C. S. Rampton

East African Veterinary Research Organization,
Muguga, P.O. Box 32,
Kikuyu, Kenya.

[1] Brotherston, J. G., *J. Comp. Path.*, **61**, 263 (1951).
[2] Cheng, S. C., and Fischmann, H. R., *Rinderpest Vaccines*, 47 (F.A.O., Washington, 1949).
[3] Macowan, K. D. S., *Rep. Dept. Vet. Ser. Kenya*, 1955, 29 (1956).
[4] Maurer, F. D., Jones, T. C., Easterday, B., and DeTray, D., *Proc. Amer. Vet. Med. Assoc.*, 1955, 201 (1956).
[5] Scott, G. R., *Bull. Epiz. Dis. Afr.*, **5**, 11 (1957).

Photo-Reactivation of Tobacco Mosaic Virus *in vivo*

Tobacco mosaic virus, inactivated *in vitro* by ultra-violet irradiation, could not be photo-reactivated by exposing plants, inoculated with the inactivated virus, to light[1,2]. However, ribonucleic acid preparations from tobacco mosaic virus could be photo-reactivated in this manner, following inactivation by ultra-violet light[1,3]. Siegel *et al.*[4] investigated the effects of ultra-violet light on tobacco mosaic virus and tobacco mosaic virus nucleic acid, at different times after inoculation of *Nicotiana glutinosa* plants, on which local necrotic lesions are formed. They interpreted their results to suggest that the nucleic

acid of tobacco mosaic virus is freed from its protein moiety during a lag period of approximately 2·5 hr. following inoculation and before the infective centre begins to increase in resistance to ultra-violet light. If this model is correct, and the nucleic acid of tobacco mosaic virus separates from the protein, then it should be possible to photo-reactivate the nucleic acid during the period when the separation occurs.

Bawden and Kleczkowski[1] reported experiments to test this hypothesis, but concluded that the photo-reactivation effects observed in their experiments could always be attributed to the effects of ultra-violet light on the 'capacity' of *N. glutinosa* leaves; capacity being defined as the ability of a leaf to support the multiplication of tobacco mosaic virus to the extent of forming local lesions. The effect of ultra-violet light on capacity can be determined by irradiating leaves prior to inoculation, and in experiments designed to study the direct inactivation of tobacco mosaic virus in leaves, the dosage of ultra-violet light must be such that the capacity remains unimpaired.

Table 1. Effect of Ultra-violet Light on the Capacity of Leaves of *N. glutinosa*

Dose (min.)	Experiment No.		
	1	2	3
2	0·97	1·00	
3	1·00	0·99	
4	0·91	0·99	
5	0·75	0·79	
6			0·56
8			0·49
10			0·24

Each figure represents the fraction of local necrotic lesions developed on detached half-leaves of *N. glutinosa* irradiated before inoculation with the *U*2 strain of tobacco mosaic virus as compared with un-irradiated half-leaves.

Table 1 shows the effect of ultra-violet light from a Philips *TUV*30 germicidal lamp (90 per cent transmission at 2537 Å.) on the capacity of detached leaves of *N. glutinosa* irradiated at 30 cm. from the lamp and immediately inoculated with the *U*2, or mild, strain of tobacco mosaic virus. Clearly, capacity is affected by prolonged irradiation prior to inoculation, and in subsequent experiments no period of irradiation longer than 90 sec. was employed. The *U*2 strain of tobacco mosaic virus was used as it is 5·5 times more sensitive to ultra-violet light than the *U*1, or common strain. Suitable killing fractions for infective centres of *U*1 tobacco mosaic virus could not be obtained without impairing capacity. *N. glutinosa* plants were inoculated by means of a paint brush with an inoculum containing 10^{-3} mgm./ml. of ultracentrifugally purified *U*2 tobacco mosaic virus in *M*/15 phosphate buffer, *p*H 7, containing 4 per cent 'Celite' as abrasive. In all experiments glasshouse grown plants were placed in a growth room 16–20 hr. before inoculation, at a temperature of 25° ± 1° C., and continuous fluorescent light of 800 ft.-candles intensity. Inoculations were carried out under these conditions, while irradiation was done under the ultra-violet lamp in the same room. Leaves were detached prior to irradiation, placed on cotton wool in shallow trays and irradiated. For each treatment 3 leaves from each of 8 plants were used; one half of each leaf was covered to serve as the unirradiated control. Leaves were retained in the light, or dark, in the growth room for 24 hr. following irradiation and then transferred to continuous light of 800 ft.-candles intensity and a temperature of 25° ± 5° C., for a further 2 days, until lesions were counted. For each treatment the lesion totals from the irradiated halves of each leaf were compared with the totals from the unirradiated halves, and since infective centres lead to the formation of local lesions the fraction of infective centres surviving irradiation was calculated.

Table 2. Photo-reactivation of Tobacco Mosaic Virus Different Times after Inoculation

Exposure after inoculation	Time after inoculation (hr.) 2	2·5	3·5	3·75	4·0	4·25	5·0
Light	0·24	0·25	0·42	0·47*	0·45	0·46	0·45	0·57*	0·61*
Dark	0·23	0·24	0·40	0·41*	0·39	0·40	0·32	0·47*	0·59*

Figures represent the fraction of infective centres of *U*2 tobacco mosaic virus on *N. glutinosa*, surviving a dose of 90 sec. ultra-violet light.

* Experiments carried out at a dose equivalent to 75 sec. ultra-violet light.

Table 2 shows the results from photo-reactivation experiments carried out at different times after inoculation. In the period from 2 to 3·5 hr. after inoculation no photo-reactivation could be demonstrated. It is clearly evident that from 3·75 to 5 hr. after inoculation photo-reactivation, as measured by the difference in the fraction of infective centres surviving irradiation in leaves exposed to light, as compared with dark, has been demonstrated. This period of photo-reactivation corresponds to the period of rapid change in sensitivity to ultra-violet irradiation shown by Siegel and Wildman[2] to commence 2·5 hr. after inoculation with the *U*2 strain of tobacco mosaic virus. Siegel and Wildman retained plants at a lower temperature, 20° C., and a higher light intensity, 1,000 ft.-candles, than those used in the present experiments. However, it is evident from the fraction of infective centres surviving when leaves are exposed to light following inoculation (Table 2) that conditions used in the experiments reported here resulted in a change in sensitivity commencing at approximately 2·5 hr. after inoculation, the same time at which Siegel and Wildman reported a change.

The demonstration of photo-reactivation supports the hypothesis that nucleic acid, free from the protein moiety, is present in the infective centre for a period of time following the inoculation of tobacco mosaic virus. Siegel and Wildman[4] suggested that the separation of protein and nucleic acid occurs during the lag period of 2·5 hr. before the infective centre begins to increase in resistance. However, in a later publication, Wildman[6] considered that the process of protein removal from the nucleic acid may occupy a period of up to 5 hr. after inoculation. Since it has not been possible to demonstrate photo-reactivation of tobacco mosaic virus until 3·75 hr. after inoculation it would appear that free nucleic acid is not present in the infective centre until this time period has elapsed. Thus it seems more realistic to propose that the separation of the protein moiety and the nucleic acid of tobacco mosaic virus in an infective centre on a leaf of *N. glutinosa* takes place following the lag period and when the infective centre is already undergoing an increase in resistance to ultra-violet irradiation.

D. J. Goodchild

Division of Plant Industry,
Commonwealth Scientific and Industrial
Research Organization,
Canberra.

[1] Bawden, F. C., and Kleczkowski, A., *J. Gen. Microbiol.*, **13**, 37 (1955).
[2] Siegel, A., and Wildman, S. G., *Virology*, **2**, 69 (1956).
[3] Rushizky, G. W., Knight, C. A., and McLaren, A. D., *Virology*, **12**, 32 (1960).
[4] Siegel, A., Ginoza, W., and Wildman, S. G., *Virology*, **3**, 554 (1957).
[5] Bawden, F. C., and Kleczkowski, A., *Virology*, **10**, 163 (1960).
[6] Wildman, S. G., in *The Viruses*, **2**, 1 (Academic Press, New York, 1959).

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, October 23

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5 p.m.—Prof. J. N. Davidson: "The Biosynthesis of Polynucleotides".* (Further lecture on October 30.)

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Discussion on "Is Automation Making Satisfactory Progress ?"—opened by Prof. A. Tustin.

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Dr. F. A. E. Pirani and Dr. G. F. Elliott: "Ballistic Missiles and Nuclear Weapons—Missile Ballistics".* (Second of seven lectures in War Studies. Further lectures on October 30, November 6, 13, 20 and 27.)

SOCIETY OF CHEMICAL INDUSTRY, PLASTICS AND POLYMER GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Mr. W. Reddish: "Chemical Structure and Electrical Properties of High Polymers".

Tuesday, October 24

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. W. Hockney and Dr. T. O. Jeffries: "The Use of Analogue Computers in Predicting the Space-Time Behaviour of Nuclear Reactors".

ROYAL INSTITUTION OF NAVAL ARCHITECTS (at the Institute of Marine Engineers, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Dr. J. E. Richards: "High Temperature Gas-cooled Reactors".

UNIVERSITY OF LONDON (at King's College, Strand, London, W.C.2), at 5.30 p.m.—Père Roland de Vaux (Jerusalem, Jordan): "Tell el-Far'ah - Tirzah—Excavating a Biblical City".*

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 5.30 p.m.—Dr. L. Brent: "Some Current Problems in Transplantation Immunity".* (Third of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

UNIVERSITY OF LONDON (at the School of Oriental and African Studies, London, W.C.1), at 5.30 p.m.—Dr. L. Dumont (Paris): "The Conception of Kingship in Ancient India, and Anthropologists' View".* (Further lecture on October 25.)

Wednesday, October 25

POLAROGRAPHIC SOCIETY (at the School of Pharmacy, 29/39 Brunswick Square, London, W.C.1), at 2.45 p.m.—Prof. M. von Stackelberg (Bonn University): "Some Special Problems in the Polarography of Indium and Tellurium".

INSTITUTE OF NAVIGATION (at the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 4 p.m.—Annual General Meeting. Dr. G. E. R. Deacon, F.R.S.: "Navigation and the Science of the Sea" (Duke of Edinburgh's Lecture).

BONE AND TOOTH SOCIETY (at the Institute of Orthopædics, 234 Great Portland Street, London, W.1), at 4.30 p.m.—Symposium on "Fluorides".

INSTITUTION OF ELECTRICAL ENGINEERS, ELECTRONICS AND COMMUNICATIONS SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. J. Halsey: "Global Communication" (Chairman's Address).

BRITISH INSTITUTION OF RADIO ENGINEERS, COMPUTER GROUP (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London, W.C.1), at 6 p.m.—Symposium on "Digital Differential Analysers".

INSTITUTION OF MECHANICAL ENGINEERS (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Sir Kenneth Hague: "Co-ordination in Engineering Research and the Conservation of Scientific and Technical Manpower" (Presidential Address).

SOCIETY OF ENVIRONMENTAL ENGINEERS (in the Mechanical Engineering Department, Imperial College of Science and Technology, London, S.W.7), at 6 p.m.—Mr. A. M. P. Brookes: "Structures and Components at Elevated Temperatures".

SOCIETY OF CHEMICAL INDUSTRY, FOOD GROUP—NUTRITION PANEL (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Meeting on "Foods Low in Salt".

SOCIETY OF INSTRUMENT TECHNOLOGY, DATA PROCESSING SECTION (at Manson House, 26 Portland Place, London, W.1), at 7 p.m.—Mr. W. H. Baker and Mr. P. Huggins: "Mechanising Factory Shop Floor Form Filling".

BRITISH PSYCHOLOGICAL SOCIETY, MEDICAL SECTION (at the Roya Society of Medicine, 1 Wimpole Street, London, W.1), at 8.30 p.m.—Mr. Emanuel Miller: "Personal and Social Approaches to Adolescents' Problems".

Thursday, October 26

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 10.30 a.m.—Symposium on "Biochemistry and Nutrition" under the leadership of Prof. R. A. Morton, F.R.S., to celebrate the Centenary of the Birth of a former President of the Society, Sir Frederick Gowland Hopkins, O.M., 1861–1947.

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. A. S. G. Curtis: "The Capabilities of a Living Cell".*

INSTITUTION OF ELECTRICAL ENGINEERS (joint meeting with the BRITISH SECTION of the SOCIÉTÉ DES INGÉNIEURS CIVILS DE FRANCE, at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. P. M. J. Ailleret: "A Comparison between Generation and Transmission Problems in Great Britain and France".

UNIVERSITY OF LONDON (at the London School of Hygiene and Tropical Medicine, Keppel Street, Gower Street, London W.C.1), at 5.30 p.m.—Dr. Henry Harris: "The Transfer of Information from Nucleus to Cytoplasm".* (Fourth of seventeen lectures on "The Scientific Basis of Medicine" organized by the British Postgraduate Medical Federation.)

Friday, October 27

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 4 p.m.—Prof. L. S. Penrose, F.R.S.: "Mongolism".*

ROYAL ASTRONOMICAL SOCIETY (in association with the GEOLOGICAL SOCIETY, at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5 p.m.—Geophysical Discussion on "Interpretation of Geophysical Surveys". Chairman: Dr. W. Bullerwell. Speakers will include Prof. J. M. Bruckshaw, Dr. M. H. P. Bott, Mr. G. Tait and Mr. A. N. Thomas.

ROYAL INSTITUTION (at 21 Albemarle Street, London, W.1), at 9 p.m.—Prof. M. A. Ellison: "Cosmic Rays from the Sun".

Friday, October 27—Saturday, October 28

INSTITUTE OF BIOLOGY (in the Lecture Hall of the Royal Geographical Society, 1 Kensington Gore, London, S.W.7), at 10 a.m. daily—Symposium on "The Better Use of the World's Fauna for Food".

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

RESEARCH ASSISTANT (Animal Behaviour) (graduate in psychology or zoology) IN THE DEPARTMENT OF PSYCHOLOGY—The Registrar, The University, Leicester (October 27).

SCIENTIFIC OFFICER (with a good honours degree in chemistry or botany) IN THE WEEDS SECTION, to carry out research on the adsorption, mobility and persistence of herbicides in soils—The Secretary, National Vegetable Research Station, Wellesbourne, Warwick (October 28).

ASSISTANT LECTURER (with a science qualification and preferably a medical qualification) IN PHYSIOLOGY—The Secretary, St. Mary's Hospital Medical School (University of London), Paddington, W.2. (October 30).

SENIOR LECTURER or LECTURER (with medical qualifications registrable in this country) IN VIROLOGY; and a SENIOR LECTURER or LECTURER (with medical qualifications registrable in this country) IN IMMUNOLOGY—The Registrar, The University, Manchester 13 (October 30).

ANALYST (with at least a second-class degree in chemistry or biochemistry or considerable experience in analytical chemistry) IN THE DEPARTMENT OF CHEMICAL PATHOLOGY—The Registrar, The University, Leeds 2 (October 31).

ASSISTANT LECTURER (with special qualifications in experimental physics, aeronomy or astronomy) IN APPLIED MATHEMATICS—The Secretary, Queen's University, Belfast, N. Ireland (October 31).

LECTURER IN BIOLOGY—The Principal, Bede College, Durham (October 31).

LECTURER (with a major interest in elasticity or the theory of plasticity) IN MECHANICAL ENGINEERING—The Registrar, Manchester College of Science and Technology, Manchester 1 (October 31).

RESEARCH ASSISTANT (graduate in physics or electrical engineering) IN THE DEPARTMENT OF PHYSICS, to investigate microwave radiation from plasmas—The Registrar, University College of Wales, Aberystwyth (October 31).

SENIOR LECTURER (with a good grounding in biochemistry and clinical medicine or clinical pathology, and preferably experience of a hospital biochemical laboratory) IN CHEMICAL PATHOLOGY—The Registrar, The University, Leeds 2 (October 31).

CHAIR OF GEOLOGY in the University of Malaya, Kuala Lumpur—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Malaya and London, November 1).

DEMONSTRATOR (graduate who intends to read for a higher degree) IN PHARMACOGNOSY—The Registrar, The University, Nottingham (November 1).

ASSISTANT LECTURER IN PHYSICS—The Registrar, Manchester College of Science and Technology, Manchester 1 (November 4).

ASSISTANT (with a degree in science and/or agriculture (preferably honours), and interest in grassland husbandry and an aptitude for investigational and research work) TO THE ADVISORY OFFICER IN GRASSLAND HUSBANDRY—The Secretary, West of Scotland Agricultural College, Blythswood Square, Glasgow, C.2 (November 7).

SENIOR LECTURER or LECTURER (qualified in applied mathematics) IN MATHEMATICS)—The Registrar, University College of North Staffordshire, Keele, Staffs (November 7).

LECTURER or ASSISTANT LECTURER (with special interests in the geography of the Americas) IN GEOGRAPHY—The Registrar, The University, Southampton (November 11).

VOKES RESEARCH FELLOW (preferably with a special interest in fluids and elastic theory) IN THE DEPARTMENT OF MECHANICAL ENGINEERING—The Secretary and Registrar, The University, Southampton (November 11).

EXPERIMENTAL OFFICER or SENIOR EXPERIMENTAL OFFICER (with H.N.C., a degree or similar qualification, and preferably experience with transistor circuits and pulse equipment) IN THE DEPARTMENT OF PHYSICS, to develop and construct special electronic devices required for experimental work on a tandem accelerator—The Registrar, The University, Manchester 13 (November 13).

SCIENTIFIC OFFICER or SENIOR SCIENTIFIC OFFICER (honours graduate with appropriate training and research experience), to work as a member of a team undertaking research on hill sheep nutrition—The Secretary, Hill Farming Research Organization, 48 Palmerston Place, Edinburgh 12 (November 13).

LECTURER (preferably with qualifications and/or experience in palæontology or stratigraphy) IN GEOLOGY at the Royal College, Nairobi—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (November 18).

READER IN FLUID MECHANICS at Imperial College—The Academic Registrar, University of London, Senate House, London, W.C.1 (November 20).

SENIOR RESEARCH ASSISTANT TO THE IMMUNOPATHOLOGY AND FLUORESCENCE MICROSCOPY RESEARCH GROUP, to work in the University Department of Pathology on immunological and histological methods—The Secretary, The University, Aberdeen (November 20).

SENIOR LECTURER IN PHYSIOLOGY at the University of Sydney, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia and London, November 25).

LECTURER IN ZOOLOGY at the University of Adelaide, Australia—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1 (Australia, November 30).

SENIOR LECTURERS/LECTURERS IN THE SCHOOL OF PHYSIOLOGY within the Faculty of Medicine, University of New South Wales, Australia—The Agent-General for New South Wales, 56–57 Strand, London, W.C.2; and The Appointments Section, The University of New South Wales, Box 1, Post Office, Kensington, New South Wales, Australia (November 30).

ASSISTANT PROFESSOR (POULTRY GENETICS) (with a Ph.D. or equivalent in poultry genetics), to develop a research programme in poultry genetics and to carry some teaching responsibility—Head, Department of Animal Science, University of Alberta, Edmonton, Alberta, Canada (January 1).

AGRICULTURAL CHEMIST (male, under 35, with a degree in agriculture with chemistry as a major subject, or a science degree with postgraduate experience in soil or agricultural chemistry) IN THE DEPARTMENT OF LAND UTILIZATION, Swaziland, to develop an agricultural chemistry section at Malkerns Research Station, organize routine analysis of soil and plant material, and to conduct an investigation into fertility problems in soils, particularly leached types of tropical soils—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.213/141/01/T.

ASSISTANT EXPERIMENTAL OFFICER (graduate, or holding H.N.C. or an equivalent qualification, or, if under 22, G.C.E. "A" level, O.N.C., etc., or equivalent, and interested in both chemical analysis and working with animals) IN THE CHEMISTRY DEPARTMENT, to assist in studies of mineral metabolism in cattle—The Secretary, National Institute for Research in Dairying (University of Reading), Shinfield, Reading, quoting Ref. 61/15.

ASSISTANT PROFESSOR IN PHARMACOLOGY—Prof. M. F. Murnaghan, University of Ottawa, Ottawa, Ontario, Canada.

BIOCHEMIST or CHEMIST (with a research degree and experience in the chemical industry involving research on new products and processes and their technical or commercial development, and preferably experience in the virology and antibiotics fields), for work concerned with the development and commercial exploitation of inventions in the public interest—The Director of Administration, National Research Development Corporation, 1 Tilney Street, London, W.1.

CHIEF LABORATORY TECHNICIAN—Head of the Department of Applied Physics, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

FISHERIES OFFICER (with a university degree in science with experience in up to date fishing methods, and the storage, marketing and distribution of fish) IN THE DEPARTMENT OF AGRICULTURE AND FISHERIES, Bermuda, to undertake the development of all aspects of the fishing industry in Bermuda—The Director of Recruitment, Department of Technical Co-operation, Carlton House Terrace, London, S.W.1, quoting RC.289/13/01/T.

HEAD (with an honours degree in mathematics of a British university and at least three years experience of teaching) OF THE MATHEMATICS DEPARTMENT—The Headmaster, Pocklington School, West Green, Pocklington, Yorks.

LECTURER (with some knowledge of logical design) IN COMPUTING—The Secretary, Northampton College of Advanced Technology, St. John Street, London, E.C.1.

PHYSICIST, Basic grade (with a first- or second-class honours degree), for routine and development work in hospital physics—The Secretary, Lambeth Hospital, Brook Drive, London, S.E.11.

POSTGRADUATE STUDENT (recent graduate with a good degree in physics or physical chemistry) IN THE DEPARTMENT OF PHYSICS, to research under the supervision of Dr. J. G. Powles on some aspect of magnetic resonance—The Registrar, Queen Mary College (University of London), Mile End Road, London, E.1.

RESEARCH ASSISTANT (honours graduate) IN PHYSICS—Clerk to the Governors, Woolwich Polytechnic, London, S.E.18.

RESEARCH ASSISTANT (with a good honours degree in physics) IN PHYSICS, for work in the field of vacuum physics—Head of the Department of Physics, The Polytechnic, Regent Street, London, W.1.

RESEARCH ASSISTANT (with graduate or equivalent qualifications and appropriate experience in electronic instrument design and construction), to take part in a space research project under Dr. T. R. Kaiser. The work will involve the development of instruments for installation in rockets and satellites—The Registrar, The University, Sheffield.

RESEARCH FELLOW IN PHYSICAL CHEMISTRY, for work with Prof. M. C. R. Symons on the study of unstable intermediates, including spectrophotometric and electron spin resonance techniques—The Registrar, The University, Leicester.

RESEARCH SCHOLAR, and a RESEARCH FELLOW IN THE DEPARTMENT OF CHEMISTRY, for work on aluminium–nitrogen and aluminium–phosphorus polymers—The Registrar, The University, Leicester.

SENIOR PHYSICIST (graduate in physics, preferably with honours and normally with five years experience of hospital physics or with radio-elements) IN THE RADIOTHERAPY DEPARTMENT—The Secretary, Lambeth Hospital, Brook Drive, London, S.E.11.

SENIOR RESEARCH FELLOWSHIP at the University of Melbourne available in any one of the academic subjects taught there—The Secretary, Association of Universities of the British Commonwealth (Branch Office), Marlborough House, Pall Mall, London, S.W.1; or Dean of Graduate Studies, University of Melbourne, Parkville, N.2, Victoria, Australia.

REPORTS and other PUBLICATIONS

(not included in the monthly Books Supplement)

Great Britain and Ireland

Colonial Development and Welfare Act. Return of Schemes made and of Loans approved under the Colonial Development and Welfare Act in the period from 1st April, 1960, to 31st March, 1961. Pp. 5. (House of Commons Paper No. 240.) (London: H.M. Stationery Office, 1961.) 3*s.* 6*d.* net. [23]

Colonial Development Corporation, Annual Report and Statement of Accounts for the year ended 31st December 1960. Pp. vi+8. (London: H.M. Stationery Office, 1961.) 5*s.* net. [25]

Vertebrate Locomotion: Proceedings of a Symposium held at The Zoological Society of London, Regent's Park, London, N.W.1, on 8th November, 1960. Edited by J. E. Harris. (Symposia of The Zoological Society of London, No. 5.) Pp. v+132+7 plates. (London: The Zoological Society of London, 1961.) 32*s.* 6*d.* [25]

British Association for Commercial and Industrial Education: Education and Training in France: Report of a Visit made by the BACIE West Midlands Group in May 1960. Pp. v+51. (London: British Association for Commercial and Industrial Education, 1961.) 10*s.* [25]

Other Countries

The Carlsberg Foundation's Oceanographical Expedition Round the World 1928–30, and previous "Dana" Expeditions. "Dana" Report No. 53; A Contribution to the Life History, Biology and Geographical Distribution of the Bonefish, *Albula vulpes* (Linnaeus). By Elizabeth C. Alexander. Pp. 51. (Copenhagen: Andr. Fred. Høst & Son, 1961.) 15 Danish kr. [18]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1475-D: Geology and Occurrence of Ground Water at Jewel Cave National Monument, South Dakota. By C. F. Dyer. Pp. iii+139–157. 15 cents. Water-Supply Paper 1499-A: Water Resources of the Providence Area, Rhode Island. By H. N. Halberg, C. E. Knox and F. H. Pauszek. Pp. iv+50+1 plate. Water-Supply Paper 1536-B: Water Levels near a Well Discharging from an Unconfined Aquifer. By Irwin Remson, S. S. McNeary and J. R. Randolph. Pp. iii+27–39. 15 cents. Water-Supply Paper 1536-C: A Simple Method for Determining Specific Yield from Pumping Tests. By L. E. Ramsahoye and S. M. Lang. Pp. iii+41–46. 15 cents. Water-Supply Paper 1539-F: Aquifers in Melt-Water Channels along the Southwest Flank of the Des Moines Lobe, Lyon County, Minnesota. By Robert Schneider and Harry G. Rodis. Pp. iii+11. 15 cents. (Washington, D.C.: Government Printing Office, 1961.) [25s]

United States Department of the Interior: Geological Survey. Water-Supply Paper 1539-I: Evaluation of Bank Storage Along the Columbia River between Richland and China Bar, Washington. By R. C. Newcomb and S. G. Brown. Pp. iii+13. 15 cents. Water-Supply Paper 1540-B: Concentration Method for the Spectrochemical Determination of Minor Elements in Water. By William D. Silvey. Pp. iii+11–22. Water-Supply Paper 1592-A: Resistance Coefficients and Velocity Distribution, Smooth Rectangular Channel. By H. J. Tracy and C. M. Lester. Pp. iv+18. (Washington, D.C.: Government Printing Office, 1961.) [25s]

National Academy of Sciences—National Research Council. Nuclear Science Series, Report No. 36: A Cross-References Index of Radiochemical Teaching Experiments Applicable to Chemistry. By Gregory R. Choppin. Pp. v+50. (Publication 905.) (Washington, D.C.: Office of Technical Services, Department of Commerce, 1961.) 1 dollar. [25s]

Annals of the New York Academy of Sciences. Vol. 92, Article 3: Pavlovian Conference on Higher Nervous Activity. By Nathan S. Kline, Gregory Razran and 23 other authors. Pp. 813–1198. (New York Academy of Sciences, 1961.) [25s]

Smithsonian Contributions to Astrophysics, Vol. 5, No. 10: Sampling Dust from the Stratosphere. By Paul W. Hodge. Pp. ii+145–152. (Washington, D.C.: Government Printing Office, 1961.) 20 cents. [25s]

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